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Review

Bio-Recovery of Metals through Biomining within Circularity-Based Solutions

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Abstract: Given the current highest demand in history for raw materials, there is a growing demand for the recovery of key metals from secondary sources, in order to prevent metal depletion and to reduce the risk of toxic discharges into the environment. This paper focuses on the current nature-based solutions (i.e., biomining and bioleaching) applied to resource recovery (metals) from solid matrices. Biomining exploits the potential of microorganisms to facilitate the extraction and recovery of metals from a wide range of waste materials as an interesting alternative, replacing primary raw materials with secondary material resources (thus improving metal recycling rates in the context of the circular economy). Special attention was paid to the analysis of metal biomining from a process sustainability perspective. In this regard, several supporting tools (e.g., life cycle assessment, LCA), developed to assist decision-makers in the complex process of assessing and scaling-up remediation projects (including biomining), were discussed. The application of LCA in biomining is still evolving, and requires comprehensive case studies to improve the methodological approach. This review outlines the fact that few studies have focused on demonstrating the environmental performance of the biomining process. Also, further studies should be performed to promote the commercial opportunities of biomining, which can be used to recover and recycle metals from solid matrices and for site remediation. Despite some important disadvantages (poor process kinetics; metal toxicity), biomining is considered to be a cleaner approach than conventional mining processes. However, implementing it on a large scale requires improvements in regulatory issues and public acceptance.

Keywords: bioleaching; metal bio-recovery; metal recycling and circularity; nature-based solution; sustainability

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1. Introduction

The 17 Sustainable Development Goals (SDGs) adopted by the United Nations in 2015 and included in the 2030 Agenda for Sustainable Development represent a global action plan for people, the planet, and prosperity, in order to end poverty and to protect the planet and human health. For example, Goal 12 is concerned with ensuring sustainable consumption and production models, which are crucial for sustaining the livelihoods of current and future generations. A scenario that predicted a population of 9.8 billion by 2050 estimated that it will require almost three planets to provide the natural resources needed to support today's lifestyle. Also, one of the strategic sustainable development targets—SDG Target 3.9—aims to minimize the number of deaths and illnesses from hazards associated with soil pollution and contamination by 2030. Furthermore, SDG Target 15.3 has the objective of combating desertification and restoring degraded land and soil, while Target 12.4 encourages the more effective management of chemicals and waste by reducing their discharge to air, water, and soil, in order to minimize their negative impact on human health and the environment [1].

Soil is a complex ecosystem with a crucial significance to terrestrial biodiversity and human agriculture. Unfortunately, soil quality is continuously affected by physical and chemical degradation due to erosion, nutrient depletion, and pollution [2]. The impact of anthropogenic activities on soils has been widely discussed in several publications [3]. The main soil degradation processes include soil erosion, the reduction of the soil organic carbon fraction, soil contamination, the loss of soil biodiversity, acidification, and salinization [3]. Of these, soil contamination caused by industrial, agricultural, and urban activities is an important anthropogenic factor that significantly alters soil properties (e.g., overexploitation, deforestation, extensive farming, improper agricultural practices, industrial pollution, and urbanization) [4]. Hence, soil pollution is mostly linked to waste disposal, mining, oil extraction, military, storage, nuclear power plants, chemicals application (pesticides and fertilizers), and others. Soil contamination poses a considerable risk to human health. For instance, the World Health Organization (WHO) estimates a global number of deaths linked to soil pollution ranging from 0.2 to 0.8 million people per year [5]. Mortality results from the SENTIERI Project (a mortality study of residents of polluted Italian locations) revealed that, in 44 sites of national interest in Italy selected for environmental remediation, about 10,000 excess deaths during the of period 1995 to 2002 were recorded. For example, in a certain number of cases, a causal role could be attributed to environmental exposure; these included reports of neurological diseases, for which an etiological role of lead, mercury, and organohalogenated solvents was possible; renal failure mortality was suspected to be due to exposure to metals, polycyclic aromatic hydrocarbons (PAHs), and halogenated compounds; increased mortality from respiratory diseases was suspected to be due to emissions from metal industries [6].

Panagos et al. [7] explored the current situation of contaminated sites in Europe and found that "the main contaminant categories are heavy metals and mineral oil contributing jointly to around 60% in soil contamination and 53% of the groundwater contamination". Tóth et al. [8] predicted a series of detailed maps of heavy metals (As, Cd, Cr, Cu, Pb, Zn, Sb, Co, and Ni) in the topsoil of the European Union, based on the Land Use/Land Cover Area Frame Survey (LUCAS) topsoil database (EU-28 except Croatia). It seems that "one or more of the elements exceed the applied threshold concentration on 1.2 M km^2, which is 28.3% of the total surface area of the EU". According to the European Environment Agency [9], the degree of soil contamination in Europe is incompletely understood, as there are no exhaustive inventories of contaminated sites at an EU level. However, based on 2016 statistics, there were around 2.8 million potentially contaminated sites in the EU-27 where polluting activities took/are taking place, while around 300,000 contaminated sites in Europe still require clean-up [9].

In 2021, the Food and Agriculture Organization of the United Nations (FAO) and the United Nations Environment Programme (UNEP) published a comprehensive report on global assessment of soil pollution, wherein it is stated that "the management and remediation of polluted sites is necessary to protect human and environmental health" [10]. In this regard, it is specified that soil represents the major recipient of the total amount of contaminants released to the environment from industrial activities (70%); the majority of these contaminants result from metal mining (72%), followed by chemical industries, energy production, hazardous waste, and others. Also, in the United States, lead and zinc were the main pollutants released from mining activities, while, in Colorado, there are around 23,000 abandoned mines requiring rehabilitation [10]. Agriculture and Agri-Food Canada (AAFC) estimated an increase in some trace elements in soil (zinc, cadmium, copper, lead, selenium, and arsenic) of up to three times, compared with natural background levels, as a result of the application of fertilizers, manure, and municipal biosolids to agricultural soils [10]. The presence of metals in soil have raised considerable attention because of their toxic effects on humans and ecosystems, thereby also affecting food security [11,12]. Metals cannot be degraded or destroyed; they are highly toxic and persistent compounds with bioaccumulation and biomagnification properties. To date, there have been numerous physical, chemical, and biological methods applied to remediate metal-contaminated sites [11]. However, each method has both advantages and disadvantages (including some limitations in terms of cost efficiency, remediation degree, secondary pollution, public acceptance, practicability, and environmental soundness) [13]. Thus, to achieve the sustainability goals related to the SDG targets mentioned above, green and sustainable remediation (GSR) should be considered in order to maximize the environmental, social, and economic benefits of soil remediation processes. GSR may also include sustainable solutions for soil remediation that can simultaneously treat soil and recover resources to enhance the circular economy [13]. Nature-based solutions, green chemistry, and sustainability assessments are the main topics associated with GSR [12]. Nature-based solutions (NBSs) are defined by the European Commission as "solutions that are inspired and supported by nature, which are cost-effective, simultaneously provide environmental, social and economic benefits and help build resilience" [14]. In terms of metal–soil remediation, NBSs include phytoremediation (a plant-based method), microbial bioremediation (working with microorganisms), and immobilization using natural (e.g., montmorillonite and zeolite), waste-derived green materials (e.g., biochar, compost, and red mud) or green-synthesized nanomaterial's [12].

It is important to note that metals are used for various purposes that are also common in our everyday lives (e.g., nickel, cobalt, copper, zinc, silver, gold, platinum, iron, manganese, aluminum, arsenic, lead, cadmium, etc.) [15]. Some of these metals are valuable raw materials, important for a country's economy. Rare earth elements (REEs), along with cobalt, copper, antimony, lithium, and indium, are identified as critical metals owing to their source, risk, and economic significance [16,17]. For example, copper has multiple applications, including batteries, circuit boards, as a conductor of heat and electricity, plating, construction, infrastructure, and electrical and electronic equipment (EEE), while also being acknowledged as a critical micronutrient for living organisms. A scenario related to copper production proposed by Elshkaki et al. [18], considering the 2010–2050 period, estimated that the demand for copper will increase by 275–350% by 2050, while the majority of copper-producing countries will be unable to support their production by 2050, based on their actual share of world copper production. According to BloombergNEF Company (New York City, USA), around 7 million electric vehicles are currently produced worldwide, which is estimated to increase by 30% by 2040. Considering that each electric vehicle contains 85 kg of copper, it is believed that copper miners will need to double the amount of copper produced globally (20 million tons) in order to fulfil the demand for a 30% uptake in electric vehicles [19].

Metal waste (printed circuit boards, batteries, computers, mobiles, and electronic devices) as well as mine tailings/mine waste, contaminated soil, slag, dust, coal fly ash, and municipal solid waste incineration fly ash (all grouped within solid waste matrices), might be potential valuable resources for metal recovery. For instance, in addition to toxic compounds (mercury, cadmium, lead, arsenic, chromium, PAHs, and dioxins), electronic waste (e-waste) may contain some precious (e.g., silver, gold, platinum, and palladium) or valuable (e.g., copper, zinc, nickel, iron, and aluminum) metals, which can be recovered, preventing their decline [20,21]. The statistics showed that global e-waste production achieved 53.6 million tons in 2019, with a recoverable material profit estimated at USD 57 billion [22]. The Umicore smelter in Belgium (the largest pyrometallurgical plant operating with e-waste) usually processes around 350,000 tons of e-waste per year and recovers more than 100 tons of gold and 2400 tons of silver per year [23].

The recovery of metals from valuable residues is essential for supplying sources of metals/materials, by minimizing the demand for limited natural/mineral resources, as well as in terms of waste treatment by reducing environmental degradation due to disposal [24]. There are two main alternatives for the bio-extraction/bio-recovery of metals from solid waste matrices, namely biomining (or bioleaching) and biosorption. Overall, nature-based biological practices (e.g., biomining, biosorption, bioprecipitation, and phytomining) provide significant advantages over conventional technologies (e.g., pyrometallurgy and hydrometallurgy): low energy consumption, more environmentally friendly approaches, lower temperatures, avoiding metal contamination, and smaller carbon footprints [24–26].

Bio-recovery technologies imply the extraction and recovery of metals using living organisms [27]. To date, significant progress has been made in developing methodologies to recover metals from waste using mesophilic, thermophilic, and heterotrophic microorganisms [24]. The first certified biomining operation was reported in 1951 when a new bacterium, *Thiobacillus ferrooxidans*, was demonstrated to be an autotrophic iron oxidizer [28]. However, it continues to be a challenging task to find suitable microorganisms that can effectively deal with the different heterogeneous textures and compositions of waste materials. Further studies should be performed to promote the commercial opportunities of bioprocesses for recovering and recycling metals from valuable residues, as well as for site remediation [24]. This review paper aims to address five objectives: (i) a short description of metal sources in the environment and their toxicity; (ii) biomining as a new aspect of circularity of waste management; (iii) microbial consortia involved in the biomining process (a description of the technology and examples of bioleaching performed by bacteria, fungi, and cyanogenic microorganisms); (iv) case studies of successful microbial application in metals recovery; and (v) multi-objective decision-making methods exploited to select sustainable biomining/remediation strategies. It is further concluded that nature-based biological solutions (such as biomining) applied to metal extraction from solid matrices offer an opportunity to generate significant benefits from an environmental point of view.

2. Sources of Metals in the Environment and Their Toxicity

Metals are characterized by their non-degradability and persistent and bioaccumulative properties in the environment. When the maximum limits set by established standards are exceeded, they are characterized as having potential toxicity for the living ecosystem as a whole [29,30]. Moreover, they are regarded as having a bio-magnification capacity within the food chain [31]. When metals such as Cd^{2+}, Pb^{2+}, Hg^{2+}, Ag^{+}, and As^{3+} react with bioparticles in the body, they form toxic compounds for which containment is essential. Specific toxic characteristics rely on bio-magnification and elevated concentration levels [32]. Still, metals are in constant demand, as they are indispensable for building modern cities and producing advanced technological goods [17,33]. Considering these aspects, significant amounts of metals are unfortunately still being leaked into the environment as a result of urbanization, agriculture and industrial operations such as mining, fossil fuel combustion, galvanizing processes, and waste products of various natures that are produced by industries such as the automotive, electric and electronic, chemical, iron and steel, textile, and petrochemical industries [15,21,34,35]. In addition, although in considerably reduced volumes compared to those of anthropogenic processes, natural processes are also important sources of metals, and, in this case, processes such as volcanic eruptions, ocean jets, the natural decomposition of organic matter, wind erosion, natural forest fires, and bedrock weathering come under this category [34,36,37].

Threats to human health were highest for metals found in water, food, toys, paints, cosmetics, and soil [38]. Exposure duration, the route of exposure, and metal concentrations in contaminated environments are associated with human health implications (e.g., neurotoxicity, nephrotoxicity, carcinogenicity, hepatotoxicity, immunological toxicity, cardiovascular toxicity, skin toxicity, reproductive and developmental toxicity, and genotoxicity) [39]. Infants, children, and adolescents show a predisposition to metal contamination, having developmental and educational disadvantages and low levels of judgment [40]. The main identified routes of human exposure to metals are via the ingestion of contaminated food and water, as well as inhalation and absorption (through the skin) from air [41]. For example, among the metals, Pb is one of the pollutants that is toxic in nature. Considering the non-smoking population, the main source of daily intake of Pb is the dietary intake of contaminated food. However, other contributing sources to adverse effects on human health caused by Pb include packaging (the use of Pb-containing welded food and beverage cans), the use of Pb-glazed ceramic or Pb-glazed tableware, household plumbing systems containing Pb, dirt particles, and dust [42]. Li et al. [43] assessed the health risks of soil contamination with metals through vegetable consumption near a large-scale Pb/Zn smelter in central China. Out of 52 samples that were analyzed, Pb and Cd were found to have the highest impact on human health, with leafy vegetables being more contaminated than non-leafy vegetables [43].

For plants, the toxicity of metals is highlighted through three diverse pathways: they shift essential cations from their binding sites, generate oxidative stress by producing reactive oxygen species, and attach directly to the carboxyl, histidyl, and thioyl groups on proteins to interact with proteins [44]. Among the key symptoms that characterize toxicity in plants are reduced plant growth and decreased photosynthetic activity [45]. The health of animals is also under threat by excessive metals available in the food chain, which can lead to damage that impacts kidney function as well as the cardiovascular and nervous systems [46,47]. For a comprehensive ecotoxicological assessment, an investigation of the toxicity of metals needs to address both lethal and sublethal effects on individual fitness. Performance and basic traits in plants are expected to combine survival, vegetative biomass, and reproductive success as the three major components of fitness [48]. Nowak et al. [48] used *Noccaea caerulescens* (Brassicaceae) as a model plant to study local adaptation to metal, cultivated in either non-polluted or Zn-polluted conditions. The principal component analyses demonstrated that the vegetative and reproductive traits were statistically independent. The same families consistently exhibited the highest or lowest performance values in two metal-exposed dependent experimental populations investigating the effects of multi-generational environmental exposure of a plant species to contaminated environments [48]. A schematic representation of the sources and toxicological impacts of metals (e.g., divided into critical metals, precious/valuable metals, and other toxic metals) to humans, animals, and plants is shown in Figure 1.

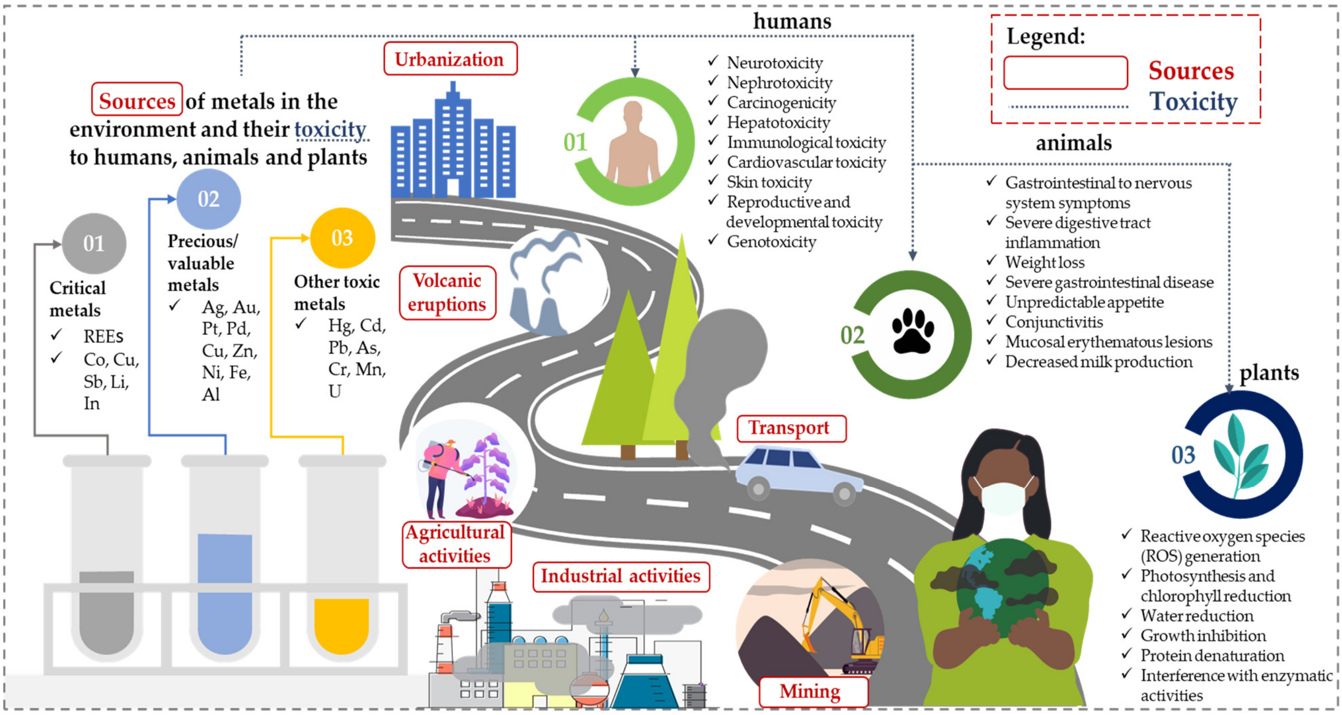

Figure 1. Contamination sources of metals in the environment causing toxicity to humans, animals, and plants (vectors designed by Freepik [49] and PNG ALL [50]).

3. Raw Critical Material Recovery

3.1. Biomining as a New Aspect of Circularity in Waste Management

Important changes in consumption models, industrial production systems, and the accelerated transition to renewable energy represent the driving factors which generated the highest demand for raw materials in history, especially for metals and minerals [51,52]. Moreover, according to data from the Organisation for Economic Co-operation and Development (OECD), the global demand for non-ferrous metals is expected to increase more than twofold from 2020 to 2060 [53], while the EU demand for rare earth elements is estimated to increase sevenfold by 2050. Thus, the gallium demand will increase 17-fold by 2050, the demand for lithium is expected to grow 21-fold by 2050, and the demand for nickel and cobalt is estimated to increase 20-fold by 2040 [54].

A new list of critical raw materials (CRMs) at the EU level was drawn up, which includes 34 raw materials, comprising three categories of materials—heavy rare earth elements (HREEs), light rare earth elements (LREEs), and platinum group metals (PGMs); among these, 16 are considered strategic raw materials (SRMs), including materials with an accelerated increase in consumption rate [55]. This list is periodically reevaluated. Generally, CRMs are considered raw materials with relevant importance from economic and strategic perspectives, which are associated with high risk for the supply chain [51,56]. This fact is mainly due to the sharp increase in their global consumption and the scarcity of metal mineral resources. Critical raw materials represent essential inputs in advanced technologies applied in priority areas, such as the transition to low carbon energy, digitization, defense capabilities, and the space industry [57]. The list of CRMs is different from one country to another. In 2020, world economic leaders such as the United States, the European Union, the United Kingdom, and India have assessed their critical minerals and have set targets to reduce their dependence on CRM imports [58].

In this general context, the EU has implemented a set of policies aimed at reducing the dependence on imports by improving supply chains from other sources, maintaining supply flows, maintaining a representative EU contribution to the raw materials sector, and reducing negative environmental impacts generated by the mining industry. Recently, the EU approved the European Critical Raw Materials Act [56], which aims to ensure a diverse, secure, and sustainable supply of essential raw materials for EU industries, to reduce the dependence on imports by improving supply chains from other sources.

In this way, a very high goal has been set by doubling the target on the circular material use rate (CMUR) between 2020 and 2030 [59], but experts have estimated that only increasing the recycling rate will not allow the achievement of this purpose. Therefore, it is imperative to improve recovery, which requires the development and application of feasible recovery processes and technologies and the optimization of an interface, with pre-processing steps for ensuring the generation of appropriate material streams [60].

Increasing the recovery capacity of metals from different waste categories represents a strategic global priority in order to decrease supply chain risks [61,62]. Biomining appears as an interesting alternative, with potential to mitigate these concerns, by exploiting microorganisms' potential to facilitate the extraction, separation, and recovery of metals from a wide range of waste [26,63,64]. Generally, biomining is considered a cleaner approach than conventional mining processes because it uses relatively low temperatures and implicitly, a lower carbon footprint [26]; also, it is considered to be a cost-effective technology [58,65]. In addition to the recovery of metals and other valuable materials from solid waste, biomining also ensures a reduction in landfill space for waste disposal and the minimization of the leaching of metals and other contaminants into the environment.

Consequently, changing production patterns by replacing the traditional linear model with the circular model, which involves decoupling from primary material resources, is crucial for achieving Sustainable Development Goals in the mining industry [52,66]. This transition involves the development of innovative processes, new technologies, and industrial synergies, especially in the emerging sectors, assuring the extraction of metals and minerals, implicitly CRM and SRM, but also applying the green remediation postmining closures, which ensures the rehabilitation of abandoned mining lands or surfaces affected by pollution and the reintegration of their use.

The application of biomining in the context of the circular economy is summarized in Figure 2, and focuses on replacing primary raw materials with secondary material resources, represented by different categories of waste. Focusing on this hypothesis, Karali and Shah [51] emphasize that, in 2050, a significant percentage of metals, especially CRMs (namely, 37–91% of the demand), could be provided from secondary raw materials. Thus, by adopting the principles of the circular economy, it may be possible to add value from mining or industrial waste and to maintain resources in use by the interconnected approaches of narrowing, slowing, and closing material loops [67], as well as through waste minimization [66]. It should be emphasized that the main challenge is the paradigm shift from considering waste as valueless flux to identifying its potential to be transformed into valuable products [68].

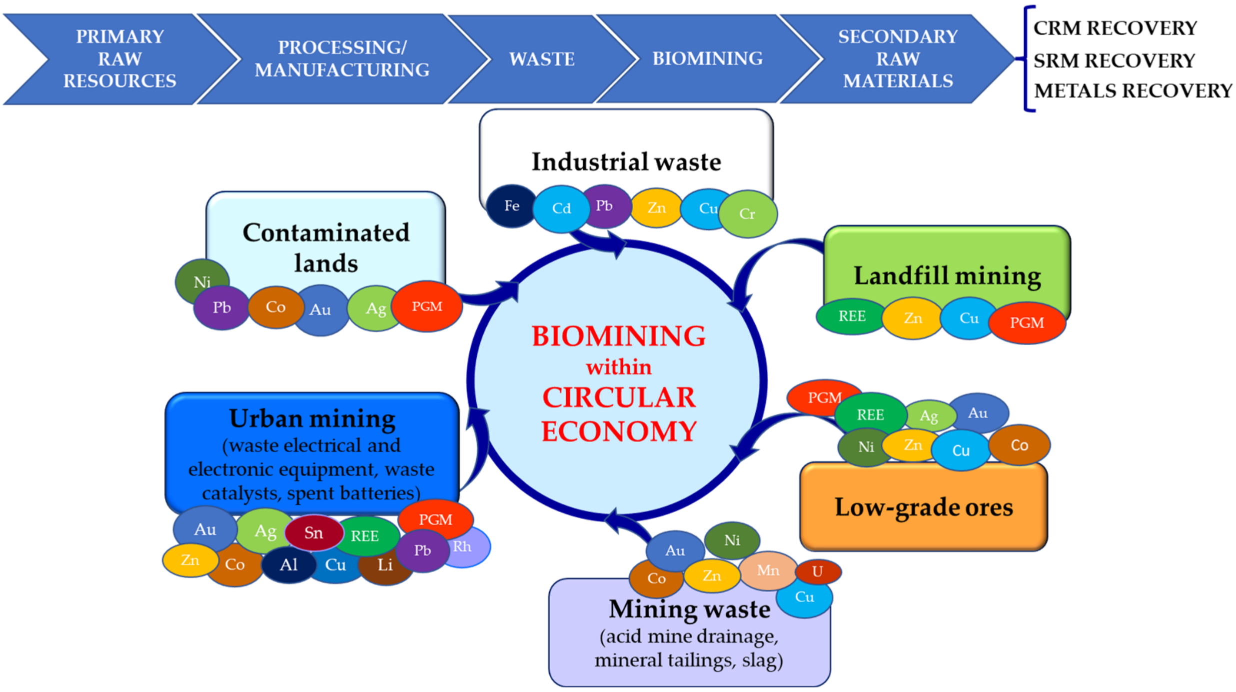

Figure 2. The contribution of biomining to the circular economy.

Thus, the implementation of sustainable and responsible mining techniques, the reduction in the intensity of material use, and the substitution of metals coupled with circular economy strategies can secure the supply chain for many metals and SRMs/CRMs, reducing, at the same time, dependence on mining activities [51,67].

However, some authors draw attention to the fact that there is a lack of appropriate value chains in the transition from a linear to a circular economic model, because, in the mining industry, these are currently missing, waste is generally outsourced, the solutions are complex and difficult to identify, and important changes are required regarding collaborations and networking [69]. Other vulnerabilities refer to the necessity of the construction of solid collection networks, the development of efficient and competitive recycling technologies, and the lack of systematized solutions based on real scenarios for closing the loops [51].

Nevertheless, an important driver in the application of a circular approach is the price of primary materials; thus, with the increase in the price of metals obtained from primary sources, the industry will move on towards the recovery of metals from secondary resources. Such a trend has been observed for cobalt, nickel, neodymium, and dysprosium, and it is expected that other priority raw materials will follow the same trend [51].

3.2. Current Status of Metal Recycling and Circularity

There is a wide interest in studies that address the dynamics of metal demands, and forecasts estimate a sharp increase by approximately two- to sixfold, depending on the metals [22,51,70]. However, Born and Ciftci [71] specified that no data are available regarding the contribution to the total estimated demand supplied by the recovery and recycling of existing metal stocks. This requires a deep analysis of the societal metabolism of metals that must include extended information about mass flows of waste materials and metals/metalloids mass fractions in waste, products, by-products, or material streams [72,73].

However, a valuable tool for assessing circularity is the end of life (EoL) recycling rate, indicating the extent to which a metal supply loop is physically closed [74]. This indicator measures the percentage of a metal input into the production system that comes from recycled waste or waste derived from the treatment of end-of-life products. The recycling rates of metals from EoL products are influenced by factors such as the quantity of materials available from end-of-life products, metal types, metal prices, the collection rate, the efficiency of the waste pre-treatment, the availability of the industrial process, and the type of end-of-life products from post-consumer waste [74–76].

Currently, in the EU, more than 90% of EoL stainless steel is recycled and integrated into new products [77]. It has been determined that the EoL recycling rate is 90% for Au, in the manufacturing processes of jewelry, ingots, and coins; in this case, an effective closed loop is attained. The EoL rate is 60% for the recovery of noble metals (Au, Ag, Pt, Pd, and Rh) from automotive catalyst waste, with a partially closed loop. An EoL recovery rate of 25% is encountered for Au, Ag, Pt, Pd, and Rh recovered from electronic product waste [74]. In the same regard, the International Energy Agency [78] reported EoL rates of 60% for nickel, 60% for Pd, 49% for Ag, 45% for Cu, 42% for Al, 32% for Zn, and 0.2% for REEs.

Similar results were reported by Srivastava et al. [63], showing high EoL recovery rates for precious metals such as platinum, palladium, gold, and silver from biomining processes. However, Saidani et al. [73] mentioned that the circularity rate of platinum from EoL catalytic converters is not optimal, with a range, in European countries, of 50–60%. The global recycling rates of Pt, Pd, and Rh, recovered from different materials, are below 35%, even if the pollution generated by the extraction of PGMs from secondary sources is lower than that generated by extraction from primary raw sources [79]. The EoL recycling rate for cobalt used in batteries worldwide is about 68% [75].

Critical metals are situated at the opposite end of the spectrum, presenting a high recycling potential; nevertheless, most of them are mainly lost, often because of technological challenges, their low concentrations in waste, collection issues, economic barriers, and unsatisfactory recovery rates [74]. Thus, the World Economic Forum [80] estimates that the recycling rate of most critical metals is less than 5%, with a total value of metals recovered from batteries of approximately 1%, and a recycling rate of Li and rare earths elements of less than 1% [17,80]. At the European level, the circularity rate of critical materials is relatively low; around 11.5% of the input materials in European industry were recovered through recycling, with an increase in the rate of less than 1%, in the decade from 2012 to 2022 [59,81].

According to Espinoza et al. [82], the urban mining rate is currently equivalent to the recycling rate of EoL products, and there is a huge gap between the amount of raw material potential in urban mining and the rate of raw materials recovered; only 20% of the amount of e-waste generated is recycled, with the rest being landfilled or incinerated.

These data indicate the need to implement sustainable alternatives to improve metal recycling rates in the context of the circular economy. Thus, it is imperative to improve specific collection strategies, to develop sufficient end-of-life infrastructures, and to subject the collected EoL products to certain advanced technologies such as biomining, which enable the efficient recovery of metals [73,74,79].

4. Biotechnologies Applied for Metal Recovery from Solid Matrices

4.1. Remediation versus Recovery

Contaminated soil represents a significant issue due to the presence of toxic compounds, including heavy metals. Different remediation technologies are applied to immobilize and transform metals into less toxic forms (e.g., ion exchange, chemical reduction, chemical oxidation, bioremediation, and phytoremediation). Along with site remediation, these contaminants can be extracted and reused, thus addressing their subsequent exploitation. Overall, resource recovery from contaminated soils may comprise the following: (*i*) the extraction of valuable metals (e.g., through biomining, biosorption, and phytomining), (*ii*) biofuels production (contaminated soil can be exploited to cultivate energy crops, which can be turned into biofuels through pyrolysis, fermentation, or gasification), (*iii*) the production of soil amendment (the polluted soil could be treated to remove contaminants and subsequently used as a soil amendment, supplying nutrients and organic matter to enhance soil quality), and (*iv*) bioremediation (the microbial degradation of pollutants in contaminated soil can result in the production of methane, which can be sequestered and exploited as a renewable energy source) [13].

The term solid matrices covers a wide variety of solid waste containing metals generated from different sources, in particular agricultural, municipal, and industrial activities. The solid matrices containing different metal concentrations are further subjected to metal mobilizing and extraction with microorganisms (the biomining process) [22]. A schematic diagram of the biomining process is provided in Figure 3.

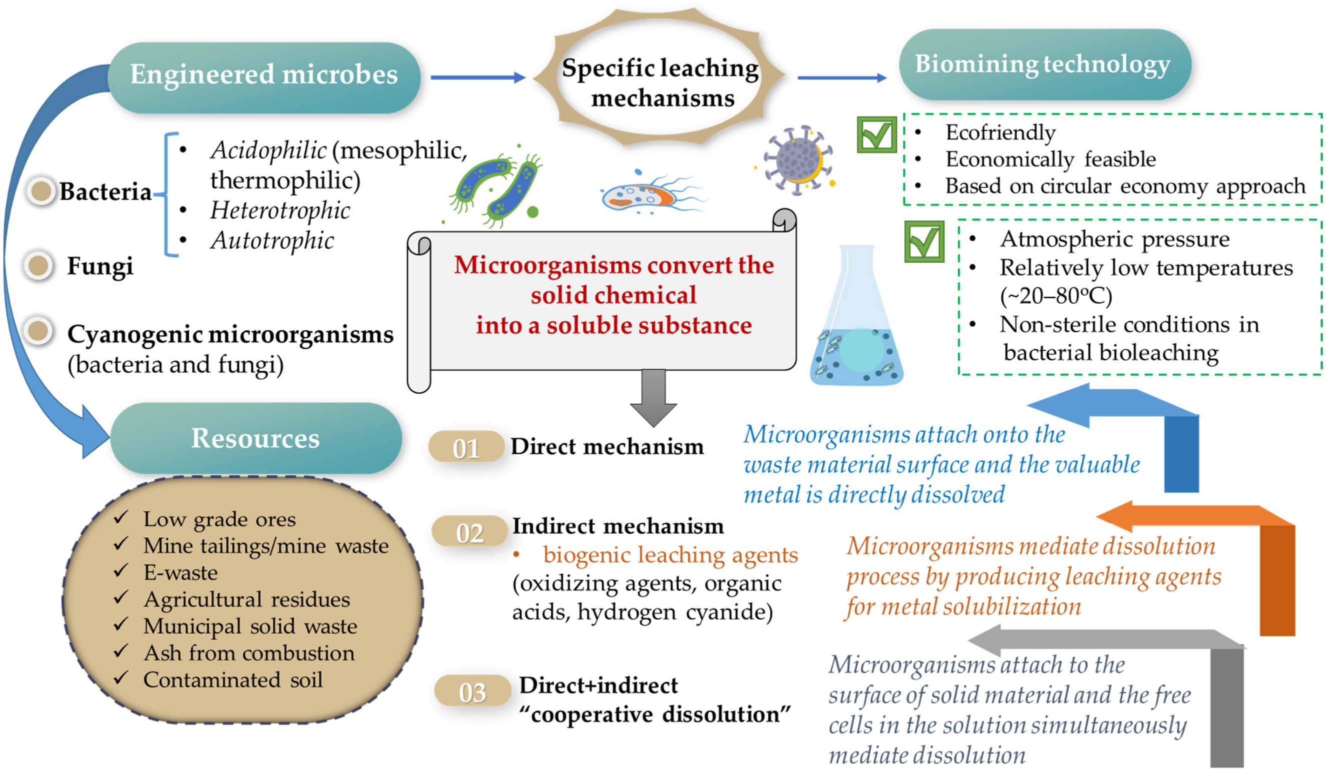

Figure 3. Schematic diagram of biomining process (after [17,26,33]).

4.2. Microbial Consortia Involved in Biomining Process

The concept of biomining covers both bioleaching and biooxidation, although the microbial responses in both cases are similar. Within the bioleaching process, the base metals (e.g., copper, nickel, zinc, and cobalt) are directly solubilized through the biological action of iron- and sulfur-oxidizing microorganisms (autotrophic), while biooxidation involves microorganisms that are employed to remove minerals that block target metals, which are solubilized in a second process. This is mainly available for the recovery of precious metals from ore and waste (e.g., gold and silver). In this case, cyanogenic microorganisms (heterotrophic) are implied, such as when bio-generated hydrogen cyanide (HCN) dissolves metals by forming a soluble metal complex such as $[Au(CN_2)]^-$ [17,24,83].

The first certified biomining operation was reported in 1951, when a new bacterium, *Thiobacillus ferrooxidans*, was demonstrated to be an autotrophic iron oxidizer and could catalyze the dissolution of pyrite in acidic liquors [28]. Earlier, in 1947, Colmer and Hinkle [84] had concluded that iron oxidation in acid mine drainage (AMD) was microbially promoted. Thus, in 1951, Temple and Colmer [28] isolated an iron-oxidizing bacterium from bituminous coal mine AMD and studied it in more detail, naming the organism *Thiobacillus*. The technology was established in 1960 by the Kennecott Copper Corporation for extracting copper from waste rock dumps (e.g., chalcopyrite, covellite, chalcocite, and bornite) at the Bingham Canyon open-pit mine in Utah, and later at Chino mine in New Mexico. Thus, the Kennecott Corporation was able to design and patent a heap leaching process for low-grade sulfidic ores and ore tailings. The original concept of the process involved spraying acidified water containing ferric iron onto heaps of ores to stimulate indigenous mineral-oxidizing bacteria. Once the solution penetrates the waste, it enters into contact with the metal sulfide in the ore, enabling the ferric iron in the solution to oxidize the metal sulfide abiotically. Consequently, the metal is mobilized in the solution [85]. Depending on the waste rock dumps, the mineral dissolution is accelerated by the ability of iron-oxidizing bacteria to generate both ferric ion and sulfuric acid (e.g., pyrite (FeS_2) and chalcopyrite ($CuFeS_2$)). In these first operations, the pregnant leach solutions containing copper-enriched liquors were collected in ponds and further processed in reactors (launders), to which scrap iron was added, resulting in an electrochemical reaction with Cu^{2+} that led to the precipitation of zero-valent copper metal (the process is called cementation). Later, heap bioleaching was improved and developed as a more refined, sophisticated, and intensive irrigation option [26,85].

The microbial solubilization of metals mainly involves chemolithotroph microorganisms, which, based on their energy source preference, oxidize inorganic compounds such as iron and sulfur to grow and develop. These microorganisms are also known as *"acidophiles"*, since they live in extremely acidic conditions (pH 1–3). Based on their optimal temperature, acidophiles are classified as mesophiles (20–40 °C), moderate thermophiles (45–50 °C), and extreme thermophiles (65–80 °C) [24,25].

Depending on their energy source, chemolithotrophs can be further categorized as *autotrophs* (which utilize inorganic matter) and *heterotrophs* (which utilize organic carbon), the latter being the least described in the literature [17,24,25,86]. Acidophiles are the most studied leaching bacteria (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, and *Leptospirillum ferriphilum*) followed by fungi (*Aspergillus niger* and *Penicillium simplicissimum*) and cyanogenic microorganisms (*Chromobacterium violaceum*, *Pseudomonas putida*, and *Pseudomonas fluorescens*) [17,87].

When more bacteria and fungi are involved, depending on their contact status, the microbial dissolution of metals can take place through different interaction reactions: direct (bacteria are directly employed, attach onto the solid waste material and simultaneously dissolve metals, with multiple reactions occurring), indirect (microorganisms facilitate metal dissolution by producing leaching agents, such as ferric ion (Fe^{3+}), sulfuric acid (H_2SO_4), organic acids (lactic, citric, gluconic, and malic acids), and hydrogen cyanide (HCN), without requiring a microbial attachment) or a combination of both indirect and direct mechanisms [17,87]. These complex interactions can be summarized by the following several mechanisms: acidolysis (an indirect leaching process that uses acidophilic chemolithoautotrophs for metal solubilization through the protonation of oxygen atoms of organic acids), redoxolysis (specific redox enzymes are secreted by microorganisms—mainly *Acidithiobacillus ferrooxidans* and *Thiobacillus thiooxidans*—and may directly oxidize or reduce highly toxic metal ions into non-toxic or less toxic forms), complexolysis (extracellular polymeric substances (EPSs) are used by microorganisms—mostly by heterotrophic cyanogenic bacteria—to form metal–complex metal ligands for the adsorption and leaching of metal ions), and bioaccumulation (the solubilization of metal ions is mediated by bioaccumulation in the mycelium via active metabolic reactions and passive adsorption) [25,88]. The biomining mechanisms, along with the reactions involved, are briefly described in the literature. Specific and complex details have already been discussed in the papers of Brown et al. [33]; Brune and Bayer [89]; Castro and Donati [24]; Gavrilescu [25]; Gulliani et al. [87]; Jerez [86]; and Tezyapar Kara et al. [17].

Once metals are solubilized from insoluble solid wastes by bioleaching, other separation methods can be employed to increase their extraction from diluted bioleachates (e.g., precipitation and solvent extraction) [33,90]. More attention has recently been paid to other potentially sustainable approaches, such as biosorption, where several types of biomass and bio-ligands are involved in binding and concentrating metal ions in the solution [22,33].

4.2.1. Bioleaching by Bacteria

Bacteria are recognized as valuable sorbents for their unique size, fast growth under controlled conditions, and adaptability to environmental conditions [91]. *Acidithiobacillus ferrooxidans* was the first microorganism studied for biomining and still remains the most and widely applied acidophiles bacterium, although some improvements have already been carried out to enlarge the range of microorganisms available (e.g., *Acidithiobacillus thiooxidans*, *Acidithiobacillus caldus*, *Acidithiobacillus albertensis*, *Acidithiobacillus sulfuruphilus*, *Leptospirillum ferrooxidans*, and *Leptospirillum ferriphilum*) [92]. *A. ferrooxidans* is uniquely capable to oxidize ferrous iron (Fe^{2+}) and elemental sulfur to ferric iron (Fe^{3+})) and sulfuric acid (H_2SO_4), respectively, while *A. thiooxidans* just oxidizes elemental sulfur and *Leptospirillum ferrooxidans/Leptospirillum ferriphilum* exclusively use Fe^{2+} iron as an energy source [17].

The impact of bacterial consortium for copper extraction from waste materials (e.g., sulfide ore, steel slag, and electroplating sludge) has been widely studied [16,93,94]. For example, Romo et al. [94] performed bioleaching tests in flasks and columns in order to identify the proper bacteria consortium for copper extraction from sulfide ore for a mining company from Chile, consisting mainly of chalcopyrite. The consortium containing *A. ferrooxidans* with *A. thiooxidans* achieved a higher removal of copper (70% in 35 days) compared to the consortium composed of *L. ferrooxidans* with *A. thiooxidans* (35% in 35 days). The test performed in bioleaching columns with the bacteria *A. ferrooxidans* and *A. thiooxidans* showed an increase in the extraction of copper from the ore compared to control sample. Parker et al. [95] concluded that the bioleaching results of previous deposits could not be extrapolated for other sites, because it is quite challenging for two sites to exhibit the same mineralogy in complete mineral compositions, meaning that generalization is infeasible. Sun et al. [96] applied a mixed acidophilic consortium (*Leptospirillum ferriphilum*, *Acidithiobacillus caldus*, and *Sulfobacillus acidophilus*) to extract copper from copper-containing electroplating sludge (CCES) (a hazardous waste). The results were compared with those obtained by chemical leaching with sulfuric acid. Microbial bioleaching showed a better efficiency (with 21.1% higher) than sulfuric acid leaching. It was interesting to find that, after the bioleaching treatment, most of the mixed metals (As, Cu, and Ni in the original CCES leachate) were removed. Thus, the bioleached residue can be viewed as a non-hazardous waste and may be considered as a raw material for the construction industry [96]. Bayat and Sari [97] compared the efficiency of microbial leaching of heavy metals from dewatered metal plating sludge with chemical leaching. For bioleaching, they applied a culture of iron-oxidizing microorganisms originating from a mining company (Turkey), mainly composed of *A. ferrooxidans*, while, for chemical leaching tests, sulfuric acid (H_2SO_4) and ferric chloride ($FeCl_3$) were used as chemical agents. The addition of $FeCl_3$ resulted in a more efficient leaching of heavy metals than the use of sulfuric acid, but it was less effective than the bioleaching using iron and/or oxidizing bacteria. At optimum conditions (pH = 2, 2% (w/v) solid content, 25 °C), after 20 days of the bioleaching process, the higher metal solubilizations obtained were as follows: 97% of Zn, 96% of Cu, 93% of Ni, 84% of Pb, 67% of Cd, and 34% of Cr. Chemical leaching with $FeCl_3$ reached lower efficiencies (79% Zn, 75% Cu, 73% Ni, 70% Pb, 65% Cd, and 22% Cr), but within a shorter time (48 h). It is clear that bioleaching achieves a higher removal efficiency (RE %) than chemical leaching in this case, but requires more time. Also, Marchioretto et al. [98] compared chemical leaching with H_2SO_4 and bioleaching with elemental sulfur (sludge amended with elemental sulfur) and ferrous iron (sludge amended with ferrous iron substrate in the form of $FeSO_4{\cdot}7H_2O$) for the solubilization of Cr, Cu, Pb, and Zn from anaerobically digested sludge. In bioleaching experiments under different conditions, Cu and Zn reached the maximum extraction yield (65.5% and 86.3%, respectively), while Cr achieved only 33.5% and Pb extraction was negligible. In chemical leaching with sulfuric acid, Zn and Cu solubilization were not very different from bioleaching, while, at a pH of 1, 100% Pb and around 72% Cr extraction yields were achieved. Finally, the authors recommended a combined treatment, with bioleaching as the first step followed by chemical leaching with sulfuric acid as the second acidification step. A positive factor of bioleaching process, in this case, is that it could reduce sludge treatment costs after metal solubilization [98]. Similarly, Dolker and Pant [99] proposed a chemical–biological hybrid system for metal recovery from waste lithium-ion batteries, resulting in an increase in Li leaching by 25% and in cobalt biosorption by 98%. Obviously, the leaching rate of Li was enhanced due to the combined effect of both chemical leaching (using citric acid, an organic chelator) and *Lysinibacillus sps*., under hybrid conditions.

In a study by Wu et al. [100], it was discovered that around 100% copper was recovered in 2 h from 5 g/L printed circuit boards (PCBs) through a bioleaching process using a microbial consortium of *Leptospirillum ferriphilum* and *Sulfobacillus thermosulfidooxidans*. Li et al. [101] reported an increase in the efficiency of the bioleaching process using mixed cultures instead of individual *A. ferrooxidans* or *A. thiooxidans* cultures. This was also the case for Rakhshani et al. [102], who obtained a higher copper recovery of 10% in the pulp density of both strains. Lewis et al. [103] showed that the addition of *L. ferrooxidans* to *A. thiooxidans* and *A. ferrooxidans* achieved a significantly higher metal recovery compared to an *A. thiooxidans/A. ferrooxidans* consortium. It seems that *L. ferrooxidans* produces greater amounts of extracellular polymeric substances (EPSs) relative to other mesophiles, while EPSs promote cell adhesion to surfaces. *L. ferrooxidans* is typically differentiated by being tolerant of higher concentrations of silver, uranium, and molybdenum, and by oxidizing pyrite at higher rates than *Acidithiobacillus* species. At the same time, it is more sensitive to copper and has a limited range of substrates that it can assimilate [104]. Hubau et al. [105] pointed out, in their study, the benefits of reprocessing mining waste through bioleaching processes. In this way, sulfide mine waste can be valorized through the recovery of valuable metals and critical raw materials, occurring simultaneously with the conversion of residues into clean minerals suitable for application in the production of cement, concrete, and construction products. In their study on recovering metals remaining in mine waste, they consider mine waste (heap leach residue, known as 'secondary ore') originating from Sotkamo mine (Terrafame, Sotkamo, Finland); they implemented bio-heap leaching for the processing of a black shale ore rich in pyrrhotite and pentlandite. For these bioleaching experiments, three bacteria consortiums were considered, mainly composed of the genera *Leptospirillum*, *Acidithiobacillus*, *Sulfobacillus*, *Acidithiobacillus*, and *Acidithiomicrobium*. Bioleaching experiments were performed on a laboratory scale, in a 2 L-batch stirred tank reactor. The results obtained in terms of leaching yield were almost 90% for Ni, 85% for Zn, and 75% for Co after 14 days [105]. A recent study by Zhang et al. [106] combined aerobic digestion and bioleaching for metal removal from excess sludge containing *Acidithiobacillus thiooxidans*, among other microorganisms. They considered two experiments: aerobic digestion followed by bioleaching (A-B process) and simultaneous aerobic digestion and bioleaching (A+B process). The bioleaching experiments were performed in a 5 L polyethylene stirred reactor with aeration, in order to maintain the normal metabolic activities of aerobic microorganisms. The results showed that the RE% of Zn, Cu, Ni, and Mn in sludge using the A+B process was higher than using the A-B process. This is justified by starting the biological leaching reaction due to the addition of sulfur at the beginning of the reaction in the A+B process. The RE% of Zn, Cu, Mn, and Ni were 87.9%, 63.3%, 69.3%, and 58.2%, respectively. An important conclusion of the study is that the combination of aerobic digestion with the bioleaching process provided a high potential for sludge treatment for its application on land, since the process reduced the risk of metal migration and enabled subsequent disposal. Abraham et al. [107] applied a new *Bacillus licheniformis* strain isolated from soil in a two-step bioleaching process for the leaching of heavy metals from electronic wastes (printed circuit boards (PCBs)). After 21 days of treatment, the authors concluded that lead and nickel were completely removed. The strain was also able to bioaccumulate 98.6% of copper, 64.6% of lead, and 57.3% of nickel. A novel combination of bioleaching and persulfate (PDS) for the removal of heavy metals from metallurgical industry sludge (from China) containing iron-oxidizing bacteria was proposed by Chen et al. [108]. The bioleaching experiments were performed on a laboratory scale in 1 L flasks with 700 mL of sludge, using 5% (v/v) inoculum and 10 g/L (w/v) $FeSO_4 \cdot 7H_2O$, at 30 °C and 150 rpm. After 18 days, bioleaching alone reached the following RE%: 70% Cu, 83.8% Zn, 25.2% Pb, and 76.9% Mn. In the combined experiment using bioleaching and PDS, an optimal additive dosage of $K_2S_2O_8$ of 8 g/L was added to the bioleaching process after 6 days. After 1 h, the removal rates of four heavy metals reached 75.1% for Cu, 84.3% for Zn, 36.7% for Pb, and 81.6% for Mn. It can be observed that the RE% was slightly increased. The major advantage of this new combination was that the treatment cycle was clearly shortened from 18 to 6 days + 1 h [108].

4.2.2. Bioleaching by Fungi

Fungi are heterotrophic species which may act as efficient metal accumulators due to their high surface area–volume ratio and high tolerance to metals [91]. *Penicillium simplicissimum* and *Aspergillus niger* are the most prevalent fungal species used in solid waste bioleaching, probably due to their ability to develop at multiple pH ranges (pH 2.5 to pH 10–12) and to provide a faster leaching rate over bacterial bioleaching. Fungal species have the ability to produce high amounts of organic acids (citric acid, oxalic acid, maleic acid, and gluconic acid) by employing organic carbons (glucose and sucrose) as an energy source, which further allow metal dissolution [17,24]. Fungal bioleaching may include the following mechanisms: acidolysis, complexolysis, redoxolysis, bioaccumulation, and even biosorption [17,109]. There have been several reports of successful metal bioleaching using fungi, but at a minimal capacity compared to bacteria [109]. For instance, Sedlakova-Kadukova et al. [110] showed that bioaccumulation was the main process for lithium extraction from lepidolite by *A. niger* (77% of the total extracted Li accumulated in the biomass). At the same time, fungal bioleaching by *A. niger* was faster (40 days) than the bacterial consortium comprising *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* (366 days), but with a lower extraction efficiency [110]. Although there are several successful results for the tolerance of fungi to metals on a laboratory scale, there is no available information on their application on an industrial scale [25,109]. On a laboratory scale, the fungal bioleaching of metals was mostly reported in the cases of solid electronic scraps [111]; mine tailings [112]; solid waste (e.g., soil samples and fly ash) [113]; and spent catalysts [114]. Studies showed that fungal species are able to successfully remove metals from soil. For instance, Liaquat et al. [115] isolated five fungal species (*Aspergillus sclerotiorum, Aspergillus aculeatus, Komagataella phaffii, Trichoderma harzianum,* and *Aspergillus niger*) from contaminated mining soil from Nanjing, China, and evaluated their tolerance to and bioaccumulation capacity of Cd, Cr, and Pb. The authors discovered a novel species as a mycoremediation agent—*Komagataella phaffii*—with a better tolerance and bioaccumulation capacity for metals compared to the other species tested. Khan et al. [113] showed that *Aspergillus niger* was the best strain for the removal of metals (Cd and Cr) from industrial metal-contaminated soil (its bioaccumulation efficiency was 98% for Cd and 43% for Cr), followed by *Penicillium rubens* (with a 98% bioleaching potential for Cd). Amiri et al. [116] provided an interesting study on the bioleaching of spent catalysts using *Penicillium simplicissimum* in batch mode, as well as considering one-step, two-step, and spent medium bioleaching. The maximum efficiency of *P. simplicissimum* was recorded during two-step bioleaching (100% of W, 100% of Fe, 92.7% of Mo, 66.43% of Ni, and 25% of Al) at 3% *w*/*v* optimum pulp density. Some studies have revealed that mixed fungal cultures efficiently extract metals from low-grade electronic waste. For example, Xia et al. [117] demonstrated the feasibility of extracting metals from waste printed circuit boards (PCBs) using mixed fungal cultures (*Purpureocillium lilacinum* and *Aspergillus niger*) in a 3 L stirred tank reactor with 8% (*w*/*v*) pulp density. Alavi et al. [118], by applying a co-fungus medium of *Aspergillus niger* and *Aspergillus tubingensis* to bioleach metals from cellphone batteries, obtained the following metal recovery rates: 52% (Co), 95% (Li), 95% (Mn), 77% (Al), and 72% (Ni), at a pulp density of 10 g/L, 30 °C, and 140 rpm. Sinha et al. [119] proposed a novel hybrid process by combining bioleaching (using the isolated Cu-leaching strain *USCT-R010*) as a recovery step and biosorption (using dead biomass of *Aspergillus oryzae* and Baker's Yeast) as a purification step, followed by electrotreatment to recover copper from waste printed circuit boards. Using this combination, a 92.7% Cu recovery rate was achieved.

Although several studies demonstrated the tolerance of filamentous fungi (*Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, and *Trichoderma*) to various metals (Cd, Cr, Co, Pb, Zn, Fe, Ni, Al, and precious metals—Ag, Au, and Pt—to a lower extent) [22], the application of fungal bioleaching on an industrial scale still remains a challenge, due to the slow kinetics, pulp density, and high cost of growth media [114].

4.2.3. Bioleaching by Cyanogenic Microorganisms

Cyanidation is an efficient technology commonly employed in gold metallurgy to recover gold from mineral ores in combination with electrochemical processes. Approximately 90% of significant gold-producing mines worldwide still apply cyanide for gold and mining, in spite of the fact that cyanides are highly toxic and are known to affect human health and the environment. The management of waste streams contaminated with cyanide and its derivatives has turned into an environmental challenge. In this regard, an alternative approach to the cyanidation process, applied to recover precious metals (Au and Ag) and platinum group metals (Pd, Rh, Os, Ir, and Ru), is being explored. This environmentally friendly approach uses cyanogenic microorganisms which are able to both produce and consume cyanide ions and to further dissolve metals from solid wastes, especially from low-grade ore and e-waste [120]. The metabolization of cyanide through the process will reduce the potential risks of human exposure and environmental contamination [121]. Cyanogenic microorganisms are heterotrophic strains (many of them being part of the soil microflora) that use organic carbon as an energy source, but they also may need amino acids like glycine in the production medium to enhance cyanide production. These organisms comprise several bacteria (e.g., *Chromobacterium violaceum*, *Bacillus megaterium*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and few fungal (*Clitocybe* sp., *Polyporus* sp., *Marasmius oreades*, and *Stemphylium loti*) and algal (*Chlorella vulgaris*) species [17,120].

C. violaceum was commonly exploited for the recovery of metals from e-waste (especially gold), mainly due to its cyanide-producing abilities, which are better than other cyanogenic microorganisms. However, it cannot be properly considered for industrial bioleaching applications, due to restrictions on its growing conditions (it is mostly found in tropical and subtropical regions) [122]. At the same time, it is believed that, overall, cyanide production is not high enough for industrial scale-up, suggesting the necessity for additional research into the engineering of microorganisms to produce higher cyanide capacities or even the isolation of new indigenous strains from contaminated sites that are able to produce cyanide in excess [120]. In this regard, Kumar et al. [122] isolated several native bacterial strains from an abandoned gold mine to extract metals from waste printed circuit boards (PCBs). Under optimal conditions (pH = 9.0, 10 g/L pulp density, 30 °C, 5 g/L glycine concentration), *C. violaceum* was able to recover Cu (87.5%) and Au (73.6%), while native *B. megaterium* SAG1 showed relatively similar results (72.7% for Cu and 66.6%

for Au). It is therefore believed that native species can metabolize high levels of toxic compounds and have the potential to growth under adverse conditions, as stated by the authors [122]. Other solutions to enhance cyanide production were associated with the optimization of operational parameters, the amelioration of bioreactor design, the optimization of medium constituents, a two-step bioleaching model where microbial growth occurs in the absence of the waste, hybrid methods (a combination of physical, chemical, and biological methods), and even the production of genetically engineered strains [120,121]. Although significant progress has been made, bio-cyanidation has been only applied on a laboratory scale (where the gold recovery rate is still very low, around 73%). It is believed that the above-mentioned efforts will reinforce the cyanide generation and improve gold leaching efficiency.

A summary list of microorganisms and their metal recovery efficiencies from different solid matrices through the bioleaching process is provided in Table 1.

Table 1. Bioleaching of metals from solid matrices: operating conditions and metal extraction efficiencies.

Microbial Group	Solid Wastes	Metal Recovery (%)	Operating Conditions	References
Bacteria				
Mixed *A. ferrooxidans* with *A. thiooxidans* Mixed *L. ferrooxidans* with *A. thiooxidans*	Copper-Sulfide ore	Cu: 70% Cu: 35%	- 250 mL Erlenmeyer flasks containing 5 g copper sulfide ore+ 90 mL medium + 10 mL bacterial inoculum; - Agitation: 170 rpm; - Temperature: 30 °C; - Time (days): 35; - pH = 2.3.	[94]
Mixed acidophilic microorganisms *Leptospirillum ferriphilum*, *Acidithiobacillus caldus*, and *Sulfobacillus acidophilus*	Copper-containing electroplating sludge (CCES)	Cu: 84.3%	- 500 mL shake flasks containing 250 mL basalt medium + 50 mL of fresh mixed consortium with 15 g CCES; - Agitation: 180 rpm; - Temperature: 45 °C; - Time (days): 10; - pH = 1.8–2.38.	[96]
Microbial consortium of *Leptospirillum ferriphilum* and *Sulfobacillus thermosulfidooxidans*	Printed circuit boards (PCBs)	Cu: 93.4%	- 250 mL Erlenmeyer flask with 10% inoculum, cultivated at the initial pH of 0.9, 42 °C, and 200 rpm with 5 g/L PCBs; - Time (days): 9; - Pulp density: 100 g/L.	[100]
Acidithiobacillus ferrooxidans and *Acidithiobacillus thiooxidans* isolated from native excess activated sludge (individual and mixed consortium)	Activated sludge	Mixed culture: 98.32% Cu and 98.60% Zn *A. ferrooxidans:* 95.98% of Cu and 96.49% of Zn *A. thiooxidans:* 95.87% of Cu and 96.83% of Zn.	- 500 mL Erlenmeyer flasks containing 200 mL distilled water with 5% (*w*/*v*) sludge and culture medium; - pH = 4 to 2 after 9 days; - Agitation: 150 rpm; - Time: 9 days; - Temperature: 30 °C; - *A. ferrooxidans* bioleaching system: 8.8 g $FeSO_4{\cdot}7H_2O$ and 10% (*v*/*v*) *A. ferrooxidans*; - *A. thiooxidans* bioleaching system, 2.0 g sulfur and 10% (*v*/*v*) *A. thiooxidans*; - Mixed culture of *A. ferrooxidans* and *A. thiooxidans*: 8.8 g $FeSO_4{\cdot}7H_2O$ and 2.0 g S, together with 5% (*v*/*v*) *A. ferrooxidans* and 5% (*v*/*v*) *A. thiooxidans*.	[101]
Mixed culture of *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, and *Leptospirillum ferrooxidans*	Polymetallic concentrates	In all tested conditions, copper and zinc maximum extractions were above 95% within 48 h; Optimum leaching time: 50 h.	- Two stage bioleaching process: fermentor filled with ceramic rings as biofilm carriers, operated in batch mode, and inoculated with the substrate-adapted cultures; - Pulp solids density: 5–20%; - Initial iron concentration: 0–15 g/L; - pH = 1.9; - Temperatures: 25, 35, and 50 °C; - Agitation: 300 and 700 rpm; - Time: 0–190 h.	[103]

Table 1. *Cont.*

Microbial Group	Solid Wastes	Metal Recovery (%)	Operating Conditions	References
Mixed bacterial culture of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*	Waste from metal ore-mining processes/soil contaminated with metals	50% Zn and 19% Fe	- 250 mL Erlenmeyer flasks containing 10 g of sterilized contaminated soil, 90 mL of 0K medium (pH 1.7), and 10 mL of cell suspension; - Temperature: 30 °C; - Agitation: 150 rpm; - Time: 42 days.	[123]
Acidithiobacillus ferrooxidans	Light emitting diode (LED) waste	Adapted *A. ferrooxidans* had higher metal bioleaching rates than the non-adapted strain: 84% Cu, 96% Ni, and 60% Ga. The highest amount of LED powder tolerated by *A. ferrooxidans* was 20 g/L.	- 250 mL Erlenmeyer flask; - The adaptation of cell culture was carried out in five steps of 5, 10, 15, 20, and 25 g/L LED powder, respectively; - Temperature: 29 °C; - pH = 2; - Agitation: 140 rpm; - Time: 30 days.	[124]
Pure and mixed cultures of moderately thermophilic bacteria *Sulfobacillus thermosulfidooxidans* and acidophilic heterotroph *A1TSB*	Electronic scrap/printed circuit boards (PCBs)	Maximum bioleachability: washed electronic scrap with mixed consortium of metal-adapted culture: 89% Cu, 81% Ni, 79% Al, and 83% Zn	- 250 mL Erlenmeyer flasks with 10% scrap concentration under different experimental conditions; - Temperature: 45 °C; - pH = 1.2–2; - Time: 18 days.	[125]
Consortium of autotrophic bacteria *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*	Abundant Li ore (lepidolite)	Bioleaching yield of almost 9% Li	- 250 mL Erlenmeyer flasks containing 190 mL of nutrient rich or poor medium + 10 mL of adapted bacterial consortium + 10 g/L crushed lepidolite; - Temperature: 30 °C; - pH = 1.5; - Agitation: 160 rpm; - Time: 366 days.	[110]
		Fungi		
Aspergillus niger	Abundant Li ore (lepidolite)	Bioaccumulation of Li into the biomass was observed: 77% of the total solubilized Li Lowest bioleaching yield of 0.2% Li	- 250 mL Erlenmeyer flasks containing 200 mL of standard liquid bioleaching media + 10 g/L crushed lepidolite; - Prior to leaching, the medium and mineral were sterilized; - Temperature: 21 °C; - pH = 5.1; - Agitation: 160 rpm; - Time: 366 days.	[110]
Aspergillus niger (M1DGR), *Aspergillus fumigatus* (M3Ai), and *Penicillium rubens* (M2Aii)	Industrial contaminated soil	All isolates (*A. fumigatus*, *Penicillium rubens*, and *A. niger*) showed higher efficiency for Cd removal with 79%, 98%, and 98% in Sabouraud dextrose broth (SDB) medium *A. niger* and *A. fumigatus* showed higher efficiency for Cr (43% and 69%) in SDB medium.	- A two-stage bioleaching process: 250 mL flask containing three different media (yeast peptone glucose (YPG), Sabouraud dextrose broth (SDB), and CM) + 2.5 g of sterilized contaminated soil sample; - Agitation: 130 rpm; - Temperature: 30 °C; - Time: 72 h.	[113]
Aspergillus sclerotiorum (A1), *Aspergillus aculeatus* (E1), *Aspergillus niger* (G03), *Komagataella phaffii* (WS), and *Trichoderma harzianum* (Y1)	Contaminated mining soil	The better bioaccumulation capacity was exhibited by *K. phaffii*: Cd (25.23 mg/g); Cu (21.63 mg/g); Pb (20.63 mg/g).	- About 0.8 g of fresh biomass was transferred to 20 mL Potato Dextrose Broth (PDB), supplemented with different metal concentrations (50, 100, 150, and 200 ppm); - pH = 5; - Temperature: 25 °C; - Agitation: 150 rpm; - Time: 7 days.	[115]
Mixed fungal culture of *Purpureocillium lilacinum* and *Aspergillus niger*	Waste PCBs	Extraction efficiency: 56.1% (Cu), 15.7 % (Al), 20.5% (Pb), 49.5% (Zn), and 8.1% (Sn).	- 3 L stirred tank reactor with 8% (*w*/*v*) pulp density of sterile waste PCBs; - Temperature: 30 °C; - Agitation: 300 rpm; - Air flow rate = 500 mL/min; - pH = 5.	[117]

Table 1. *Cont.*

Microbial Group	Solid Wastes	Metal Recovery (%)	Operating Conditions	References
Aspergillus fumigatus	Soil mine tailings	One-step process at 1% (w/v): Pb (56%), As (62%), Fe (58%), Mn (100%), Zn (54%) Two-step process at 1% (w/v): Pb (88%), As (32%), Fe (45%), Mn (58.4%), Zn (31.3%).	- One-step process: sterilized 250 mL Erlenmeyer flasks containing 1 mL spore suspension inoculated + 100 mL sucrose medium + sterilized tailings samples at 1%, 2%, 4%, or 8% (w/v); - Two-step process: 250 mL Erlenmeyer flask containing 100 mL sterilized sucrose medium inoculated + 1 mL of fungal spore suspension +, after 15 days, sterilized tailings samples at 1%, 2%, 4%, or 8% (w/v) were added; - Temperature: 30 °C; - Agitation: 150 rpm; - pH = 2.75 to 8.16; - Time: 40 days.	[126]
Aspergillus niger	Power plant residual (PPR) ash	At maximum pulp density of 9 (%w/v): V (83%) and Ni (30%).	- Spent-medium bioleaching: bubble column bioreactor fermentation experiments followed by leaching tests in Erlenmeyer flasks with various pulp densities of PPR ash (1, 2, 3, 5, 7, and 9 (%w/v)); - Time: 7 days; - Temperature: 60 °C; - Agitation: 130 rpm; - Optimum conditions in bubble column: Aeration rate of 762.5 (mL/min), sucrose concentration of 101.9 (g/L), and inoculum size of 40 (mL/L).	[127]

5. Case Studies of Successful Microbial Applications in Metals Recovery

5.1. Reusing Bio-Recovered Metals in Circular Economy

The analysis of the material flow shows that, currently, approximately 15–25% of copper, 5% of gold, and lower amounts of zinc, nickel, cobalt, and uranium are obtained from biomining activities worldwide [63,65,128,129]. The higher rate of recycling metals such as cobalt and nickel is determined by the high prices of these primary raw materials, while other metals as lithium and manganese are usually recovered at a reduced rate, due to the lower price of these primary raw materials [51]. Also, the recycling rate is directly influenced by the availability of separation technologies, as well as by the complexity of the processes involved. For materials such as lithium, manganese, and copper, which technically could be recycled at a high rate, in practice, the effective recovery rate is low, mainly due to the difficulties of separation from waste such as spent batteries [51]. However, in the case of spent lithium-ion batteries (LIBs), bacterial and fungal leaching proved their effectiveness for the dissolution of metals from these used batteries, demonstrating a higher dissolution rate for Li than for Co [130]. Recent research has assessed the possibilities of extracting other critical materials, such as REEs and phosphorus, from ores and e-waste [33].

The application of bioleaching processes to dumped waste resulting from uranium extraction has reported recovery rates of approximately 75%, while heap leaching technology has proven the possibility of recovering 60% of the copper, over an average period of 30–48 days [131].

Adetunji et al. [132] synthesized a series of promising results from applying bioleaching processes for metals recovery to e-waste, using different techniques and microbial strains. The reported extraction efficiencies were 86% for copper, 80% for zinc, 74% for nickel, and 64% for aluminum, from electronic scrap; other works have established recovery efficiencies of 96% for copper, 93% for cobalt, 85% for zinc, and 73% for nickel following the bioleaching of metals from PCBs. However, lower extraction efficiencies were obtained for Au (28%), Ag (0.25%), and Sn (16%), and the recovery rate of Pb is also very low (0.25%) [133].

The copper bioleaching process applied to printed circuit boards (PCBs) demonstrated the possibility of 95–100% copper recovery, under specific conditions, for a period of 48 h [134]. Similar results were presented by other authors who studied biomining for the recovery of Cu from PCBs, with the efficiency of the process being 95% [135]. Studies carried out by Mäkinen et al. [136] applied iron- and sulfur-oxidizing microorganisms to the bioleaching of industrial tailings, with the aim of recovering cobalt and other metals. The results obtained in a pilot installation revealed high leaching yields for Co (87%), Zn (100%), Ni (67%), and Cu (43%), over a processing time of 10 days. A study by Bhatti [137] analyzed the bioleaching of polymetallic black shales, and showed that microbial leaching solubilized 80–90% of the total incorporated metals (U, Cu, Ni, Mn, Mo, Y, and Zn), over 15–20 days of bioleaching.

5.2. Application of Biomining Process in Metal-Contaminated Soil/Sediment

Studies have demonstrated that bioleaching is also efficient for the extraction of metals from contaminated soils (Table 2). However, the majority of studies have centered on laboratory studies without pilot-scale testing [138].

Table 2. Bioleaching of metals from contaminated soils/sediments.

Soil Type	Pollutant Type	Bioleaching Strain	Operating Conditions	Efficiency	References
Industrial soil sites (Pakistan)	Pb, Hg	Indigenous fungi isolated from soil (*Aspergillus niger* (M1), *Aspergillus fumigatus* (M3), *Aspergillus terreus* (M6), and *Aspergillus flavus* (M7))	- Lab scale: 250 mL pre-sterilized conical flasks inoculated with types of media (YPG, CYE, and SDB); - Two-step bioleaching; - Temperature: 30 °C; - Shaking at 120 rpm for 120 h.	- Pb removal rate at 99.20% and 99.30% using *A. fumigatus* and *A. flavus*, respectively; - Hg removal rate of 96% and 95.50% in the YPG medium using *A. niger* and *A. terreus*, respectively; - The highest Pb uptake efficiency: 8.52 mg/g in the YPG medium; - The highest Hg uptake efficiency: 0.41 mg/g in the CYE medium.	[113]
Industrial soil site (smeltery) (China)	Cd, Cu, Pb, Zn	*Penicillium chrysogenum* strain F1	- Lab-scale: 250 mL flask; - Two-step bioleaching; - Temperature: 28 °C; - Shaking at 120 rpm for 8 days.	- Cd: 152 mg/kg, 30.8%; - Cu: 564 mg/kg, 97.5%; - Pb: 3160 mg/kg, 32.8%; - Zn: 7812 mg/kg, 80.4%.	[139]
Industrial soil site (smeltery) (China)	Zn, Pb, Mn, Cd, Cu	Microorganism isolated from a vegetable oil sample: *Burkholderia* sp. Z-90	- Lab-scale: 500 mL flask; - Two-step bioleaching; - Temperature: 35 °C; - Shaking at 180 rpm for 5 days.	- 44.0% for Zn, 32.5% for Pb, 52.2% for Mn, 37.7% for Cd, 24.1% for Cu, and 31.6% for As.	[140]
Contaminated soil from a smelting plant (China)	Pb, Zn, Cd, Cu	*Aspergillus niger* F2	- Lab-scale: 250 mL flask; - Two-step bioleaching; - Temperatures: 25 °C, 30 °C, and 35 °C; - pH = 5, 7, and 9; - Shaking at 120 rpm for 7 days.	- At 30 °C, pH = 5, bioleaching by *A. niger* with sucrose, glucose, maltose, lactose, and starch: 69.86% for Cd, 66.57% for Cu, 64.59% for Pb, and 69.01% for Zn.	[141]
Agricultural soil (Clemson University, South Carolina)	As	*Aspergillus niger*	- Lab-scale: 250 mL flask containing 180 mL growth medium + 15 g soil + 1 mL of *A. niger* inoculum (with or without glucose as a carbon source); - One-step bioleaching; - Temperature: 30 °C; - Shaking at 200 rpm for 28 days.	- 7.9% removal of As in case of glucose/ *A. niger* treatment.	[142]
Industrial soil sites (Romania)	Cu, Pb, Cr, Ni	*Thiobacillus ferrooxidans* (TF)	- Lab-scale: 250 mL flask: TF inoculated in 9K medium * (20 mL or and 40 mL) +10 g of soil; - One-step bioleaching; - Temperature: 27 °C; - Shaking at 200 rpm for 12 h.	Cu: 29–76%; Pb: 10–32%; Cr: 39–72%; Ni: 44–68%.	[143]
Soil of smelting industry site (China)	Cd, Pb, Zn	*Aspergillus flavus*	- One-step bioleaching: fungus incubated with the medium and sterile soil in a rotary shaking incubator for 15 days; - Two-step bioleaching: pure culture of the fungus was run for 6 days, and then sterile soil was added; bioleaching experiment: rotary shaker for 9 days.	- 130 mg/L sucrose for *A. flavus* in bioleaching for 15 days (16.38% for Pb, 30.55% for Cd and 52.66% for Zn); - Cd and Zn were higher in two-step bioleaching (49.66% for Cd and 65.73% for Zn); - Optimum conditions: sucrose as carbon source, pH 7, and 30 °C.	[144]

Table 2. *Cont.*

Soil Type	Pollutant Type	Bioleaching Strain	Operating Conditions	Efficiency	References
Agricultural soil (China)	Pb, Zn, Cr, Cu, Ni, Cd	*Aspergillus tubingensis* F12	- Soil column: length of 2 cm, inner diameter of 1 cm with 1 g of soil + 20 mL of EPS solution.	- EPS adsorbed metals at a significantly higher rate than the F12 pellets.	[145]
Soil of smelting industry site (China)	Pb, Cr, Cd, Cu, Mn, Zn	Soil-originated Mn(II)-oxidizing bacteria (*Providencia* sp. LLDRA6) + $BioMnO_x$ (oxidation of Mn(II) into $BioMnO_x$ on the cell surface)	- 500 mL of leachate in 1 L Erlenmeyer flask, continuously shaking at 35 °C at 180 rpm; - Solid ratio: 1:5, 1:10, 1:15 and 1:20 (*w*/*v*).	Pb: 81.72%; Cr: 88.29%; Cd: 90.34%; Cu: 91.25%; Mn: 56.13%; Zn: 59.83%.	[146]
Soil sample collected from uranium tailings (China)	U	*Acidithiobacillus ferrooxidans* ATCC 23,270, *Leptospirillum ferriphilum* YSK, *Acidithiobacillus thiooxidans* A01, and *Acidithiobacillus ferrivorans* YL15	- Lab-scale: 250 mL flask: 15 g of sterilized soil + 150 mL culture medium + 5 g/L iron +5 g/L elemental sulfur inoculated with the four bacteria in a volume ratio of 1:1:1:1.	- 85.81% removal of U, in case of mixed consortium.	[147]
Sediment samples (Deûle Canal, France)	Mn, Zn, Cu, Cd, Pb	- Fungi (*Aspergillus niger* and *Penicillium chrysogenum*); - Indigenous microflora.	- Semi-pilot scale: 45 L air-lift bioreactors; - Temperature: 30 °C; - Fungal leaching experiment: inoculum ratio of 10% (*w*/*w*); - Bacterial leaching experiment: inoculum ratio of 5% (*w*/*w*) sulfur enrichment.	- Fungi under saccharose treatment after 45 days: 77% for Mn, 44% for Zn, 12% for Cu, 1.6% for Cd, and <2% for Pb; - Bacteria under sulfur treatment after 30 days: 72% of Cu, 85% of Mn, 91% of Zn, and 93% of Cd.	[148]
Sediment samples (Puti Lake, China)	Cu, Ni, Zn	Indigenous sludge bacteria obtained from the Hangzhou Qige Wastewater Treatment Plant	- Lab-scale: 250 mL flask (150 mL working volume); - Inoculum: 1% (*v*/*v*); - Solid ratio: 1% (*w*/*v*); - Sulfur: 3 g/L; - Initial pH: 8.0; - Temperature: 28 °C; - Shaking at 180 rpm for 9 days.	- Cu: 284 mg/kg, 74.27%; - Ni: 84 mg/kg, 35.35%; - Zn: 394 mg/kg, 69.92%.	[149]
Contaminated sediment samples from Ell Ren River (southern Taiwan)	Zn, Cu, Ni, Cr	Indigenous sulfur-oxidizing microorganisms from sediment	- Continuous bioleaching experiments: 50 L (working volume) continuous stirred-tank reactor (CSTR) agitated at 200 rpm and 30 °C; - 10% (*v*/*v*) of acclimated sulfur-oxidizing microorganisms; - Hydraulic retention time: 10 days; - Aeration rate: 12 L-air/min; - Operation time: 30 days.	-At 3–5% of sulfur dosage: Zn: 47–81%; Ni: 60–93%; Cu: 41–91%; Cr: 13–72%.	[150]
Sludge mine sediment (Czech Republic)	Fe, Zn, Cu, Pb	*Acidithiobacillus ferrooxidans*	- Bioleaching of samples in pilot plant conditions in a Bioflo/CelliGen 310 bioreactor; - Temperature: 30 °C; - pH = 2.0; - 2.5% and 4.2% (*w*/*v*) pulp densities; - Agitation: 150 rpm; - Particle size < 40–200 µm; - Time: 42 days.	- At 2.5% (*w*/*v*): Zn: 97.08%; Fe: 58.75%; Cu: 79.11%; Pb: 89.35%. - At 4.2% (*w*/*v*): Zn: 95.03%; Fe: 47.08%; Cu: 73.03%; Pb: 80.69%.	[151]

Note: yeast peptone glucose (YPG), Czapek yeast extract agar (CYE), and Sabouraud dextrose broth (SDB); 9K medium *: $(NH_4)_2SO_4$–3.0 g; KCl–0.1 g; K_2HPO_4–0.5 g; $MgSO_4{\cdot}7H_2O$–0.5 g; $Ca(NO_3)_2{\cdot}4H_2O$–0.01 g; $FeSO_4{\cdot}7H_2O$–44.2 g; EPS (extracellular polymeric substance) solution.

There are two microbial groups commonly used in the bioleaching of metal-contaminated soils, namely fungi (e.g., *Penicillium* spp. and *Aspergillus* spp.) and bacteria (e.g., *A. thiooxidans* and *A. ferrooxidans*) [138]. Under laboratory optimum conditions (pH = 5, temperature of 30 °C), Xinhui et al. [141] obtained the following bioleaching results using *Aspergillus niger* with sucrose, glucose, maltose, lactose, and starch: 69.86% for Cd, 66.57% for Cu, 64.59% for Pb, and 69.01% for Zn. In his master's thesis, Gilstrap [142] demonstrated the ability of *Aspergillus niger* to extract arsenic (As) to a certain extent from contaminated agricultural soils. The experiments were performed in 250 mL flasks inoculated with 180 mL growth medium + 15 g soil + 1 mL of *A. niger* inoculum (with or without glucose as a carbon source, 100 g/L) (one-step bioleaching). After 24 days of treatment, the experiment with glucose/*A. niger* showed a 7.9% removal of arsenic. On the one hand, the treatments without an amendment of glucose accomplished only a 1% removal of arsenic. Extending the period of the experiment, after 35 days, in the same conditions, the treatment with glucose/*A. niger* showed better efficiency (17.6% removal of arsenic through solubilization). The author also demonstrated that a large portion of the arsenic in soil was biovolatilized throughout the experiment. Therefore, in the case of As, bioleaching with *A. niger* is not quite a proper remediation method, because biovolatilized arsenic is not fully captured. This can explain the relatively lower values for the removal of As through solubilization, compared with other metals [142]. Although it was found that the metabolism and enzymatic activity of fungi are negatively impacted by arsenic, they were still effective in bioleaching heavy metals such as Cu, Pb, Zn, and Cd from contaminated soil [142]. On the other hand, Tran et al. [152] pointed out that, since acidophilic bacteria are growing in a low pH and produce sulfuric acid during their metabolism, an extremely acidic condition will be generated by the bacteria. This acidic condition may destroy microbiota and deplete soil nutrients, leading to the degradation of soil quality and productivity. Therefore, it is clear that there is a necessity to find a proper bioleaching technique, when dealing with toxic elements such as As, that can efficiently extract them from agricultural soils under neutral pH conditions and without the acidification of the soil. In this regard, Tran et al. [152] evaluated the effect of indigenous microbial consortium on the bioleaching of arsenic from contaminated soil by *Shewanella putrefaciens*. *Shewanella* spp. has a versatile metabolism and is able to grow both in anaerobic and aerobic conditions. *Shewanella* spp. is able to enhance As mobility in soil through the reduction of Fe(III) to Fe(II). The final results showed that the highest removal efficiency of As was encountered in the experiment with the bacterial combination (57.5%), followed by *S. putrefaciens* (30.1%), and the indigenous microbial consortium (16.4%).

An interesting study was performed by Tang et al. [145], who tested simultaneous in situ remediation, leaching different heavy metals (Pb, Zn, Cr, Cu, Ni, and Cd) from a soil column, using extracellular polymeric substances (EPSs) of *Aspergillus tubingensis* F12, on a laboratory scale. The authors found that EPSs excreted from *A. tubingensis* F12 were efficient remediation agents and could leach metals from soils [145]. Andrzejewska-Górecka et al. [153] tested the efficiency of bacteria from activated sludge, enriched by 1% dusty sulfur, in the bioleaching of cadmium (252 mg/kg) and zinc (957 mg/kg) from contaminated soil from an embankment in Poland. A two-step bioleaching process was used. In the first step, the bioleaching culture, based on activated sludge, was activated under conditions of shaking (120 rpm), a temperature of 24 °C, a pH of <2, and 1% sulfate concentration. In the second step, metal leaching was performed in 300 mL Erlenmeyer flasks containing 200 mL of leaching media or 1% sulfuric acid and 10 g of waste, which were shaken at 120 rpm, at a temperature of 24 °C, and aerated with compressed air. After 14 days, 66% of Zn was released, in the case of the simultaneous mixing and aeration conditions experiment. For Cd, the highest efficiency (99%) was obtained after 7 days of treatment with both mixing and aeration, while, on day 14, only 44% of Cd was released. This can be explained by involvement of a secondary sorption that has taken place in the biomass of the microorganisms or in the soil itself, as stated by the authors [153]. A mixed bacterial culture and pure culture of four strains (*Acidithiobacillus ferrooxidans* ATCC 23,270, *Leptospirillum ferriphilum* YSK, *Acidithiobacillus thiooxidans* A01, and *Acidithiobacillus ferrivorans* YL15) were tested and compared in a study on uranium removal from radioactive contaminated soil (originating from China), provided by Chen et al. [147]. The synergistic effect of mixed bioleaching with a bacterial consortium improved the uranium removal efficiency (to 85.81%). A new study was released by Zhang et al. [154] on the bioleaching of Cd, using *Aspergillus niger*, from contaminated *Helianthus annuus* L. stalk harvested from mildly to moderately contaminated soils. The experiments were performed on a laboratory scale, in shake flasks containing 5.0000 g of sunflower stalk and 100 mL of leaching medium (inoculated with *A. niger*), at 30 °C and 150 rpm, for 15 days. The best results, in terms of Cd removal rates from the plant through bioleaching, were obtained for a sucrose concentration of 100 g/L (48.43%), an initial pH value of 3 (53.73%), and an inoculation amount of 10% (46.54%). Under optimum conditions (sucrose: 76.266 g/L; inoculation amount: 10%; initial pH: 3), 67.67% of Cd was removed by the *A. niger* leaching system after 11 days. Finally, the authors recommended this direct leaching method with *A. niger*, without pretreatment, as a safe treatment in the recycling of Cd-contaminated sunflower stalks [154].

There has been less work carried out on sediments, compared to other solid waste, but some improvements have already been made in the bioleaching of toxic metal sediments. Sediments are apparently very challenging matrices, compared to mineral ores. In particular, metal contaminants are likely to bind to sediment components (e.g., through adsorption on organic molecules or association as exchangeable ions) [155]. A critical analysis of the main barriers influencing the efficiency of bioleaching as a sediment remediation strategy is provided by Fonti et al. [155]. As stated by the authors, given the relatively limited number of microbial strains that were investigated to remove metal contamination from polluted sediments, exploiting different microbes could provide new insights in this area. For example, Sabra et al. [148] performed fungal (*Aspergillus niger* and *Penicillium chrysogenum*) and bacterial (indigenous microflora) bioleaching tests to extract metals from sediments on a semi-pilot scale in a 45 L air-lift bioreactor. The authors stated that solubilization was mainly observed for those metals that were bound to the acid-soluble fraction of the sediments, namely Mn and Zn. Bacterial bioleaching with a sulfur treatment provided both better and faster results, since, in 30 days, 72% of Cu, 85% of Mn, 91% of Zn, and 93% of Cd had been solubilized. In this case, the leaching process is supposed to be closely related to the oxidation of sulfur by lithotrophic bacteria in the sediment [148]. More recently, Chen et al. [150] used a continuous bioreactor when bioleaching heavy metals from contaminated sediments (the samples were provided from Ell Ren River in southern Taiwan). In the first step, the indigenous sulfur-oxidizing microorganisms were acclimatized into an acrylic reactor stirred at 200 rpm, at 30 °C with an air flow rate of 6 L /min. In addition, they also added elemental sulfur as the substrate for the microorganisms. In the second step, continuous bioleaching experiments were performed in a 50 L (working volume) CSTR bioreactor, agitated at 200 rpm and at a temperature of 30 °C, with a solid content of 10% (w/v), 10% (v/v) of acclimated sulfur-oxidizing microorganisms, supplied with 3%, 4%, and 5% (w/v) amounts of sulfur pellets, with a hydraulic retention time of 10 days and an aeration rate of 12 L air /min. The authors obtained good results in terms of maximum solubilization efficiencies for Zn (78%), Ni (90%), Cu (88%), and Cr (68%), at a 5% sulfur dosage and after 30 days of processing time. It was interesting to find that an increase in sulfur dosage resulted in an increase in metal removal efficiency from sediments. In the end, the treated sediments were quite stable and safe for the ecosystem. The authors explained that this was possible due to the fact that, during the bioleaching process, the quantities of metals linked with exchangeable, Fe and Mn oxides-bound, carbonates-bound, and organics/sulfides-bound fractions in the sediment appeared to decrease [150].

5.3. Industrial-Scale Biomining Techniques

The extended reaction time (up to 70 days to month) and relatively low leaching efficiency (usually <60%, depending on operational conditions, microorganism type, metal type and concentration, etc.) are major constraints to the widespread application of this process on a large scale [156]. In this regard, several strategies are being used to enhance metal extraction during bioleaching [90]. The effectiveness of microbial bioleaching can be enhanced by providing an optimum pH, temperature, oxygen and carbon dioxide requirements, nutrients, and mineral substrates.

Overall, based on indirect/direct mechanisms, bioleaching engineering approaches have been scaled in practice: one-step, two-step, and spent-medium step bioleaching [17]. For example, Pradhan et al. [157] explored the bioleaching process (using *Acidithiobacillus ferrooxidans*) for the extraction of nickel (Ni), vanadium (V), and molybdenum (Mo) from spent refinery catalysts in the first step. To increase the extraction of Mo (50% in the first step), they included a second leaching step with acid and alkali leaching reagents ($(NH_4)_2CO_3$, Na_2CO_3, or H_2SO_4). Mo leaching was highly improved (99%) after the second step with $(NH_4)_2CO_3$ [157]. In another study, sequential bioleaching, with two stages of bioleaching using *A. ferrooxidans* or *A. thiooxidans*, was reported to increase the Mo extraction rate [158]. It seems that the sequential strategies enhance metal solubilization and provide a shorter-duration bioleaching process [158]. A valuable study was conducted by García-Balboa et al. [111], who applied sequential bioleaching and bio-uptake of metals from electronic scraps. Actually, the authors combined a bioleaching treatment using an acidophilic bacteria consortium (*Acidiphilium multivorum* and *Leptospirillum ferriphilum*) in the first stage, with microalgae-mediated uptake from e-scraps leachate in the second stage (two acidophilic microalgae were used: *Euglena* sp. and *Chlamydomonas* sp. strains). The recovery values of the bioleaching stage were around 100% for Cu, Co, Al, and Zn, while the two microalgae strains were able to bio-uptake Zn, Al, Cu, and Mn.

The percentage of metal bio-recovery is influenced by different variables (pH, temperature, pulp density, the particle size of the wastes, shaking speed, bioleaching duration, etc.). Since the process depends on a huge range of variables, extensive efforts have been made to obtain a proper selection of optimum experimental conditions using an experimental test design (DOE) and, to a larger extent, response surface methodology (RSM). RSM is an important statistical tool since it gives information on the interaction effects of variables with fewer experiments [159]. However, it has two significant limitations: (i) "the obtained model is accurate only within the experimental range, and extrapolation is not applicable" and (ii) "RSM is a local analysis—the developed response surface is invalid for regions other than the studied ranges of factors", as stated by Naseri et al. [159].

The scale of biomining applications is divided into three parts: laboratory scale (extensive studies are performed in shake flasks, where various operation parameters are optimized, but this is not enough to design commercial-scale reactors), intermediate scale (using a stirred reactor or column reactor), and large scale (dump leaching, heap leaching, agitated leaching using an agitated tank reactor, and in situ leaching) [17]. Of these, the most commercially used processes for the cost-effective extraction of metals are heap, in situ, and tank leaching. Finally, biomining technology has progressed considerably; it is already being applied on commercial/industrial scales, but only for different types of **ores**; biomining is used to extract copper, gold (to a larger extent), and nickel using acidophiles bacteria, predominantly in Chile, Spain, China, the USA, and Australia [17,109].

Chile is one of the world's leading countries in terms of bioleaching operations and also produces three times more copper than the world's second largest producer, the USA. It is known that Chile provides around 30% of world's copper production [160]. The majority of **copper** heap bioleaching sites using acidophiles in Chile are operated by Codelco (Chilean National Copper Corporation) and BHP Billiton. For example, Cerro Colorado mine, operated by BHP Billiton, produces 100,000 tons of copper/year. Between 2016 and 2022, Nippon Mining and Metals Company Ltd. (Tokyo, Japan) collaborated with Codelco (Santiago, Chile), and BioSigma S.p.A. (Cona, Italy) biotechnology company was formed. Since 2014, BioSigma has asserted 82 biomining-related patents [83].

Finland is one of the most important countries in terms of nickel, gold, and cobalt mining resources. Mondo Minerals (Sotkamo, Finland)—the second largest producer of talc worldwide—developed Mintek's bioleaching technology for the recovery of nickel and cobalt. The commercial plant, constructed on the site of the existing Vuonos talc concentrator plant, was designed to produce about 1000 tons of nickel and 20 tons of cobalt annually [83].

DNI Metals Inc. (Mississauga, Ontario, Canada), achieves, through bioleaching, the recovery of nickel/uranium/zinc/copper/cobalt and REE-Y-Sc-Th mineral resources from black shale polymetallic sulfides, in order to recover viable quantities of REEs [161].

In Europe, the largest known copper reserve is the German and Polish copper mining district of the Kupferschiefer ores. Several projects were involved in metal recovery from the Kupferschiefer ores using bioleaching technology, as follows: r2—R011 (Integrated Process for Innovative Extraction of Metals from Kupferschiefer Mine Dumps), FP6-Bioshale, and FP7-ProMine [93]. Bioshale was a European project co-funded by the European Commission within the 6th research framework program of the European Union [162]. Based on the Bioshale project, a heap leaching approach on the Talvivaara deposit was performed—the viability of the process was demonstrated and, currently, the Talvivaara project is at the industrial phase. The Talvivaara project (Finland) is the first world's heap leaching project for nickel recovery [163]. Some other efforts to generate scalable technologies should be emphasized. At an EU level, projects such as BIORECOVER, RAWMINA, RUBICON, and BIOCriticalMetals have been implemented, targeting specifically the extraction and recovery of CRMs, REEs, PMGs, and magnesium using specific biomining processes [164]. Thus, the RUBICON project developed a scalable bioleaching technology for the exploitation of nickel-rich oxide ores and waste, with the main goal of the recovery of nickel, cobalt, and scandium, as well as copper, manganese, zinc, and vanadium [165]. Another large project developed at the European level, H2020-NEMO—Near-zero-waste recycling of low-grade sulfidic mining waste for critical-metal, mineral and construction raw-material production in a circular economy—focused on valorizing sulfidic tailings by increasing the technology readiness level of hydrometallurgical processes, in order to extract and recover metals and critical metals. Other objectives of the project included the concentration of hazardous waste, the removal of sulfur in the form of sulfate salts, and the recovery of residual mineral fractions in processes such as the manufacture of cement, concrete, and construction products. Three exploitation mines were targeted: the Sotkamo Ni-Co-Zn-Cu mine (Finland), the Luikonlahti Cu-Zn-Ni-Co-Au-ore processing facility of the Kylylahti mine (Finland), and the Tara Zn-Pb mine (Ireland) [166]. The obtained results demonstrated the possibility of separating and recovering some metals, such as Zn, Ni, Cu, Co, REE, Mn, K, and Mg. Moreover, the (bio)hydrometallurgical processes developed for metal recovery were applied at different pilot scales, which validates the possibility of valorizing these categories of waste through biomining [167].

The use of biomining on a large scale in the Aguablanca deposit (Spain) offered the possibility of exploiting a Ni–Cu–platinum-group element (PGE) deposit, grading 0.66 wt.% Ni, 0.46 wt.% Cu, and 0.47 g/t PGE [168]. The project BioProLat—Reductive Bioprocessing for Cobalt and Nickel recovery from Laterites—implemented in Brazil focused on applying biohydrometallurgical processes to Co and Ni recovery from laterites, with the main goal of increasing the level of metal recovery from the existing mines, recovering unexploited ores, and transforming limonite reserves into valuable resources [169].

Biooxidation in stirred tanks for refractory gold ores with a high content of gold, using the BIOX® process by Metso Outotec, is probably the most significant development to demonstrate the commercial application of gold biooxidation [160]. The Metso Outotec BIOX® process has been in commercial operation for more than 30 years, with 13 successful BIOX plants commissioned worldwide, which at present cover more than 25 million ounces (781 tons) of gold production. The latest technical development within BIOX® is the MesoTherm process, which applies a combination of mesophile (40 °C) bacteria for the primary oxidation stage, followed by thermophile (65 °C) bacteria for the secondary oxidation stage [170].

Olimpiada mine (in Russia) operates one of the largest proprietary biooxidation processes in the world, for refractory gold–arsenic ore treatment using a chemolithoautotrophic microbial consortium. The technology is patented and protected by the trademark

BioNORD®. In 2017, over 30 tons of gold were produced at the Olimpiada gold recovery plant [83].

The highest quantities of heap leached copper are derived from the dry areas of the world (e.g., Australia, Mexico, United States, Chile, and Peru). Supplying enough water to compensate for evaporation and losses that occur in the heap leaching process is one of the operators' problems. Low temperature can also be an issue in metal heap leaching, since microbial activity in heaps is typically reduced in cold regions. However, cold climate heap bioleaching is relatively common and attractive for regions with long, cold winters (e.g., Canada and Finland). This is probably because there is evidence that some microorganisms can catalyze the dissolution and leaching of sulfide minerals at low temperatures (<10 °C) [171]. For example, Langdahl and Ingvorsen [172] observed that microbial pyrite oxidation rates in North Greenland (at 0 °C) accounted for 30% of the maximum leaching percentage. Zeng et al. [171] discovered in their study that an around 2 g/L copper concentration was achieved at 6 °C using *Acidithiobacillus ferrivorans* YL15. Therefore, appropriate technology must be in place to mitigate the cooling of the heap and the solution during irrigation, along with the possibility of heating during off-use periods. Talvivaara's heap project (from eastern Finland) is one of the few examples that is able to remain in operation in ambient conditions as low as −20 °C. The sulfide ores available at Talvivaara are associated with pyrrhotite, as they have the ability to release significant amounts of heat when oxidized. This heat generation makes the leaching process ideal for the sub-arctic climate of Eastern Finland, where the outside temperature can vary between −30 °C and +30 °C.

According to the analysis of the market research and consulting company Credence Research, biomining processes generated a total of USD 1.5 billion for mining companies in 2020 [173]. Given the results presented above and the economic forecasts, we can state that biomining will become a large-scale alternative for metal recovery and the market share will increase significantly, with the industry expected to reach a market value of USD 3.6 billion by 2027 [173].

6. Multi-Objective Decision-Making Methods Exploited to Select Sustainable Biomining/Remediation

6.1. Sustainable Remediation

The remediation and management actions of mine sites, soils, tailings, and land are not inherently sustainable [174], and they could generate negative consequences during the remediation process, such as the potential for secondary pollution or long-term effects related to social acceptance [13]. Some authors [13,175] highlight the importance of implementing a holistic approach to soil remediation, through developing sustainable alternatives which assess the economic, social, and environmental impacts of remediation methods. In this regard, decision-making regarding the management of contaminated soil has evolved from a rather simple and linear process into a complex procedure involving different relevant aspects for site remediation and management. The concept of sustainable remediation has attracted attention in the general context, including economic, environmental, and social considerations in contaminated site management practices [176]. Sustainable remediation has been defined by SuRF-UK "as the practice of demonstrating, in terms of environmental, economic and social indicators, that the benefit of undertaking remediation is greater than its impact and that the optimum remediation solution is selected through the use of a balanced decision-making process" [177].

The general objectives of sustainable remediation summarized according to U.S. EPA [178] are as follows: remediation targets, supporting the use and reuse of remediated land, increasing operational efficiency, reducing the pressure generated by contaminants and waste into the environment, minimizing degradation or enhancing ecological processes on site, reducing air emissions and greenhouse gas generation, minimizing impacts on water quality and the water cycle, conserving natural resources, achieving greater long-term economic benefits through investment, and increasing sustainability in pollutant removal processes [178].

The most recent concept, sustainable and resilient remediation (SRR), is an optimized model for the removal of pollutants and for the limitation of environmental damage during the clean-up process, as well as for the increase of social and economic benefits and the incorporation of resilience and climate change adaptation into contaminated site remediation design [179–181]. In addition, the objective of resilience is also underlined in the new EU Soil Health Law initiative, which establishes that, by 2050, all soil ecosystems are to be "healthy". This means that sustainable land use and remediation are becoming the new norm [182].

SRR strategies are focused on approaches that encourage resource recovery from contaminated soil, apply circular economy principles, conserve natural resources, and use specific tools to assess environmental impacts [13]. Green remediation has as a target priority the maximization of the net benefit for the environment (NEB) by analyzing both the environmental benefits and the environmental costs involved throughout the life cycle of remediation actions [183]. The criteria for selecting a viable option in the remediation and restoration process is a critical step that requires the relevant analysis of several factors depending on the site conditions, contaminant characteristics, cleanup efficiency, legislative standards, remediation costs, the required cleanup level, secondary pollution risks, remediation time, etc. [181,184].

In sustainable and resilient remediation projects, bioremediation and phytoremediation are frequently selected, involving the exploitation of the natural potential of microorganisms and plants to clean up contaminated soil and recover resources [13]. According to Vo et al. [64], the sustainable approach in the field of biomining should consider the following criteria: promoting environmental resilience, economic efficiency, the efficiency of technologies, and the availability of secondary resources. Based on data in the literature, the results of Strengths, Weaknesses, Opportunities, Threats (SWOT) analysis and techno-economic assessment indicate that biomining is a sustainable alternative for the recovery of rare metals from low-grade resources, with a low environmental impact [64].

6.2. Application of Sustainability Assessment Tools in Biomining

The analysis of the biomining of metals from a process sustainability perspective can be achieved using the support tools developed to assist decision-makers in the complex and difficult process of assessing remediation projects, such as life cycle assessment (LCA), life cycle sustainability assessment (LCSA), multi-criteria decision analysis (MCDA), cost–benefit analysis (CBA), spreadsheets for environmental footprint analysis (SEFA), sustainable remediation tool (SRT), SiteWise™, human health risk assessment (HHRA), and net environmental benefit analysis [175,185,186], which ensure the possibility of qualitative and quantitative evaluation [181,187].

Given the complexity of soil pollution phenomena and the dimensions of contaminated areas, it is generally accepted that no method can fully meet all criteria for sustainable remediation and restoration [175]. Some of the most widely used tools for assessing different remediation processes' sustainability are represented by SRT, SiteWise™, SEFA, MCDA, and LCA (not necessarily in this order). These tools were developed to calculate the ecological footprint or process impacts (depending on the tool) in different phases of the remediation, offering to the practitioners the possibility to select a sustainable technology by comparing alternative remediation and identifying opportunities, in order to minimize the impact of the method in the design or optimization phases [188,189].

The SRT tool is able to provide users the possibility to estimate sustainability indicators for specific remedial technologies [190]. This tool is developed using similar algorithms as those applied in life cycle assessment and is an integrated decision-making tool that allows users to weigh different values as a part of the selection process. SRT generates quantitative results for each selected technology, providing information on calculated sustainability metrics, sustainability values for gas emissions, technology costs, energy and water consumption, risks and changes in resource service of restored soil and groundwater, land value, and ecological functioning [185].

The SiteWise™ tool (Vancouver, Canada) is applied to assess the environmental footprint of soil remediation technology, and involves a consistent set of metrics—greenhouse gas emissions, energy consumption, water consumption, air emissions, resource consumption, safety and accident risk [191]—identifying all the activities involved in the implementation of the technology [192]. The SiteWise™ tool is a flexible tool, which presents the advantage of subdividing scenarios into modules, thus offering the possibility of application for the assessment of contaminated megasites [187]. The tool was successfully applied to quantify the environmental footprint of remediation projects for soil contaminated with lead [184]. Relevant research on the tool and its applications were conducted by Xiao et al. [187], who used the tool to quantify environmental footprints and identify emission reduction pathways in megasites contaminated with toxic metals (As, Hg, Sb, Co, Pb, Cd, Zn, and Ni) and organic compounds [187]. Other studies selected the SiteWise™ and SRT tools to develop an ecological and sustainable strategy to remediate a site contaminated with metals and organic pollutants; the results revealed that phytoremediation is recommended to treat the majority of the site, while solidification/stabilization was the sustainable alternative selected for the areas with high contaminant concentrations [192].

Similarly to SiteWise™, the SEFA tool is a quantitative evaluation tool that characterizes the environmental footprint produced the remediation activities for a specific remedial process [188]. A study conducted by Khan et al. [193] analyzed environmental footprints using the SEFA algorithm for different remediation scenarios for a hypothetical contaminated site. The environmental impacts associated with different technologies applied to the remediation project of an aquifer below an industrial site were calculated using the tools SiteWise™ and SEFA [194]. Both tools confirmed that bioremediation was the option with the lowest environmental impact. However, from our knowledge, there is limited research on the application of these tools for the quantitative environmental footprint assessment of biomining processes.

Multi-criteria decision analysis (MCDA) is a useful decision-making tool for evaluating different options or alternatives considering different criteria (remediation effect, remediation cost, remediation time, environmental impact, and societal impact). It establishes the preferences for evaluating, prioritizing, or selecting available alternatives expressed in terms of multiple criteria, which are usually conflicting. This method was applied to evaluate remedial alternatives considering also the effect of applied technologies for remediation and time of remediation [195].

Life cycle assessment (LCA) is defined by the standards ISO 14040 and ISO 14044, and includes four main phases of application, as presented in Figure 4 [13,196–199]. LCA allows for the conversion of generated emissions and consumed resources into a quantifiable impact category. Usually, LCA is able to quantify the secondary and tertiary impacts of remediation projects [13]. Most LCA studies on the biomining process are developed on a laboratory scale, in order to explore the potential impact on the environment and to identify weak points in the process. The purpose of the LCA application is to establish the potential for a large-scale implementation of the process in question and its possible impacts on the environment [36,197] assessing all the stages of the remediation process, from the preparation of the contaminated site to the use of microorganisms in the remediation process, the recovery of metals, and the management of the resulting biomass and waste. In this way, the tool guides further technological progress by providing support on possible directions for process design improvement [197,200].

Di Maria et al. [201] conducted a comprehensive sustainability analysis, using a life cycle sustainability assessment (LCSA), of the application of bioleaching, on a pilot scale (NEMO technology), for the recovery of Cu, Zn, Ni, and Co from tailings generated in a sulfide mine located in Sweden. The implementation of the project will generate 22% higher climate changes, but will reduce the use of mineral resources by 98% compared to extraction from primary resources. The social analysis highlights the benefits regarding employee compensation and access to local resources, while the economic analysis shows that the viability of the process is determined by the purity of the recovered cobalt. However, the authors mentioned that the study had some limitations generated by the transition from pilot scale to the industrial scale.

A recent study elaborated by Diwan et al. [202] compared several scenarios considering the management and storage of municipal waste by applying LCA; the results showed that sorting, biomining, composting, and landfilling with energy recovery generates a lower impact on the environment than the scenario in which the waste is stored directly. The assessment highlighted the fact that the scenarios which involve leachate recirculation, landfill gas recovery, and biomining lead to the reduction of global warming potential, eutrophication potential, and acidification potential.

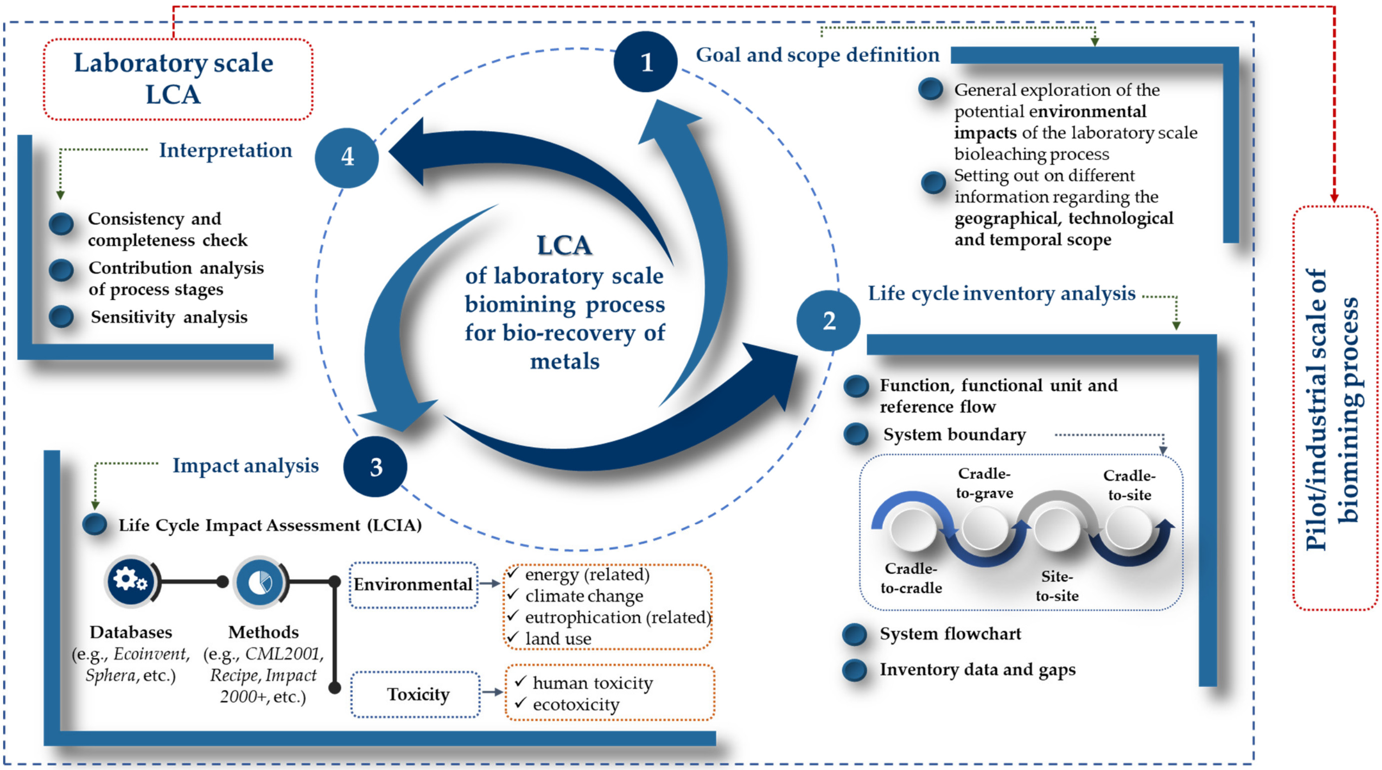

Figure 4. Upgrade of the biomining process for the bio-recovery of metals from laboratory scale to pilot/industrial scale using the four steps of LCA methodology.

The comparison of traditional treatment alternatives with a tailing desulfurization flotation process (in South Africa), using LCA, showed an important decrease in human toxicity, eco-toxicity, urban land occupation, and natural land transformation impacts; it showed an increase in climate change, fossil fuel depletion, and terrestrial acidification impacts, which were attributed to electricity production [203].

An LCA study was conducted by Sun et al. [204] to estimate the environmental impact of a bioleaching method for spent Zn-Mn batteries in a small pilot experiment; the results underlined that the main environmental impacts categories were represented by human toxicity and marine ecotoxicity, which may be attributed to direct metal emissions during the mechanical cutting and crushing process [204]. Also, an LCA study was carried out to compare the bio-hydrometallurgical and conventional mechanical–pyrometallurgical processes to extract Cu from waste PCBs. The authors indicated that bioleaching had a lower environmental impact than pyrometallurgical processing [205].

Stamp et al. [206] performed an LCA study on Umicore Precious Metal Refining (Belgium), which recovers 17 different metals from end-of-life products and from by-products generated by the non-ferrous industry. The analyzed system is very complex and dynamic and is characterized by multi-input/multi-output processes, changing feed compositions, and time lags, which make the typical LCA representation problematic. The assessment reveals that a smelter–refinery cannot be characterized by static, assignment inventory models, which makes the choice of allocation rationality arbitrary. Consequently, marginal parameterized models are necessary, even if more time and specific data are required. The authors emphasize the challenges but also the lack of accuracy of the LCA tool in the case of complex large-scale industrial systems. However, the sensitivity of the results related to the subjective and ambiguous allocation choices presented in this study has been noticed and discussed by different works [197].

A case study conducted to compare different alternatives for the indium recovery process from e-waste showed that the bioleaching process had a higher impact on the environment than the analyzed chemical processes, which was attributed to the long duration of the biological process and its high electricity consumption. However, it should be mentioned that the study outlines that an LCA during the design project phase presented significant uncertainty, related to the lack of data about and knowledge of the process [207]. Different studies [200,204,207], carried out on pilot or laboratory scales, demonstrated that electricity and fossil fuel consumption generate the dominant environmental impacts.

It can be summarized that most of the LCA studies carried out have shown that this tool provides relevant information for future cleaner productions, for the integration of solutions that focus on the recovery of valuable materials from waste/soil, and for more sustainability-oriented decision-making [201].

However, even if life cycle assessment (LCA) is successfully used on a large scale as a tool for analyzing industrial systems, the application of LCA in biomining is still evolving, and requires comprehensive case studies to improve the methodological approach [208,209]; the main challenges identified were defining system boundaries, harmonizing methods and indicators for impact assessment, allocation, data availability, and data quality [197,206,207]. Recently, methodologies based on the life cycle thinking (LCT) approach have been considered to be more comprehensive assessment methods to facilitate decision-making, because they can obtain a more relevant perspective on all aspects of mining projects throughout the entire life cycle [208].

The three basic instruments aimed at the different dimensions of sustainability within the process life cycle are: environmental life cycle assessment (E-LCA), economic life cycle costing (LCC), and social life cycle assessment (S-LCA) [196,201]. Nevertheless, some experts [208,210] have pointed out that most of the existing methodologies are currently insufficient in integrating the entire spectrum of elements from a mining site and the consequences of mining activities on environmental, economic, and social aspects.

To sum up, for the development and integration of future solutions, further studies in this sector should be encouraged, with a priority being to obtain solid scientific evidence for all aspects of sustainability decision-making, as well as the development of tools that can fulfill the GSR principles.

7. Final Conclusions

Biomining—a proven technology for metal solubilization and subsequent extraction from solid waste matrices—is an economical and environmentally friendly methodology with several advantages over chemical methods (higher removal efficiencies for metals, low costs, environmentally friendly, low energy requirements, and easier management).

In conclusion, biomining is not easy to operate and requires process optimization in terms of temperature, pH, agitation, the addition of substrates for proper bacterial growth, the chemical form of the metal, the type of microbial strain, leaching time, solid content, pulp density and particle size, inoculum volume, and/or CO_2/O_2 supply. The optimization of the appropriate bioprocess parameters influencing the growth and metabolism of microorganisms is crucial for improving the effectiveness of the biomining process. Further, in order to improve the adaptation of microorganisms under different conditions, genetically engineered microorganisms could be created; this would allow for the development of versatile organisms for the optimal removal and recovery of metals from the environment. Acidophiles are the most studied leaching bacteria, followed by fungi and cyanogenic microorganisms (although the scientific community has not yet succeeded in developing an optimized metal recovery technology using fungi, as this is not commercially available).

Several studies on a laboratory scale demonstrated that biomining is an efficient method for metal removal from contaminated soil. The process is likely more complicated due to the involvement of a secondary sorption that can occur in the biomass of microorganisms or in the soil itself. Moreover, there was less research on sediments, compared to other solid wastes. Sediments are apparently very challenging matrices compared to mineral ores, since metal contaminants are likely to bind to sediment components (e.g., adsorption on organic molecules, association as exchangeable ions).

Depending on the solid matrices type, the toxicity of waste (e.g., e-waste) could be a huge challenge for the biomining process, because it disturbs the growth of organisms to produce metabolites. There are some situations when industries release waste effluents and sludges into the environment without proper treatment beforehand. These industrial wastes may contain high amounts of toxic metals (e.g., Cd, Cr, Co, Zn, and Pb). A positive factor of biomining process, in this case, is that it could reduce waste effluents/sludge treatment costs after metal solubilization.

Extensive and rigorous work at a laboratory level has already demonstrated the possibility of resource recovery using biomining technology. Unfortunately, the long duration of the process and its relatively low recovery yields are some critical shortcomings affecting the large-scale (commercial) recovery of metals from various solid matrices. The commercial industrial methods used for the cost-effective extraction of metals are dump, heap, in situ, and tank leaching. From our knowledge, the industrial application of biomining technology has only been implemented to extract copper, gold, nickel, and uranium for different types of ores (from a mining field) using acidophilic bacteria. Nevertheless, there is still much room for further progress and research in the field of industrial applications.

Further, we should bear in mind that only satisfying the standard limits for waste disposal is not a reliable condition for metal disposal in the environment. Metal removal should be strongly recommended, as the accumulation of metal loads at a disposal site represents a significant threat to the environment. The question that arises now is thus: is remediation valid without further metal (bio)recovery? Nevertheless, considering biological organisms' ability to recover metals from the environment is an essential step in mitigating the risk of critical thresholds for metals, along with detoxification. On the other hand, the issue of waste generation from the extractive and processing industries must be addressed (e.g., the mining industry is one of the largest generators of waste in the world). Therefore, it is crucial to intensify the recovery of secondary materials by applying circular economy principles. The application of biomining in the context of the circular economy is mainly focused on replacing primary raw materials with secondary material resources, represented by different categories of waste. Unfortunately, the processing and valorization of waste is not a priority activity for large mining companies, as solutions are complex and difficult to identify, requiring important changes regarding collaborations and networking. There are also some uncertainties regarding the markets for materials recovered from landfills, since they are mixed with significant amounts of other waste that need to be separated and processed.

It is obvious that resource recovery by means of biomining technology will bring significant advantages for public health, economic growth, and environmental sustainability. In this regard, the analysis of eco-efficiency, economic feasibility, recovery performance, and systems durability must be addressed. These can be achieved by considering different related tools, such as environmental footprint analysis (e.g., SEFA, SiteWise™), qualitative and semi-quantitative sustainability assessment tools, and life cycle assessment tools (e.g., LCA). Even if these tools were predominantly applied to demonstrate the efficiency of site decontamination using bioremediation technologies, it is believed that further progress will also be encountered in the case of the biomining process. This review buttresses that little research has focused on demonstrating the environmental performance of the biomining process.

Biomining offers considerable possibilities for recovering metals from different solid matrices (e.g., industrial waste, soil, agricultural waste, e-waste, etc.), thus balancing the costs of site remediation and operation through waste valorization. This can also minimize the environmental impact associated with raw material extraction, since potentially toxic elements are not released into the environment and are instead recovered within the circular economy. Currently, increasing the recovery capacity of metals from different waste categories represents a strategic global priority, in order to decrease supply chain risks.

Generally, biomining is considered a cleaner approach than conventional mining processes, because it uses relatively low temperatures, has a lower carbon footprint, and does not produce hazardous by-products. Despite some important disadvantages (poor process kinetics and metal toxicity—a major challenge for microorganisms), the attractiveness of biomining continues to be its undoubted environmental friendliness, compared with processing operations such as pyrometallurgy and hydrometallurgy. However, implementing it on a large scale requires regulatory approval and public acceptance.

Obviously, further research should be developed to determine the most feasible option for metal recovery based on a specific biotechnological process, along with the assessment of recovery alternatives as a strategic approach for a more sustainable and competitive economy. Further work in the improvement of bioleaching technologies may include a wide range of approaches, such as the following: the application of microbial strain associations, innovations regarding the process conditions and stages of the bioleaching process, the use of growth stimulators, process optimization, adopting hybrid systems by combining biological methods with physico-chemical methods, and the application of genetically engineered microorganisms. However, such approaches must be validated through techno-economic feasibility analyses and assessments of the processes, taking into account their environmental and social impacts (e.g., LCA) and recovery performances.

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