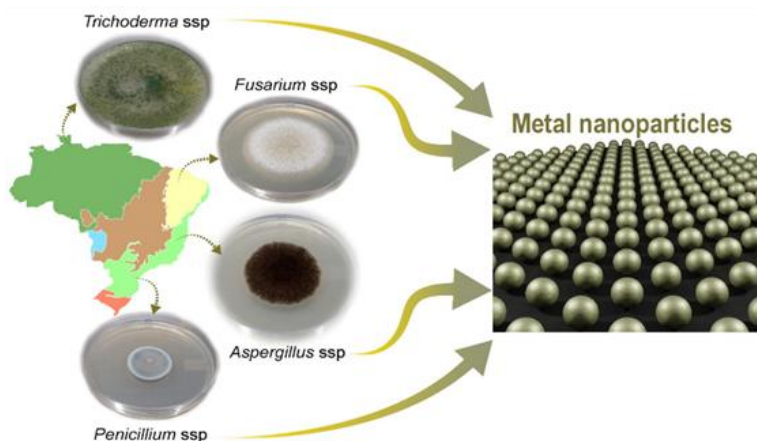


Overview of emerging research of fungi isolated from the Brazilian environment for the biosynthesis of metal nanoparticles

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Abstract

Brazil's rich biological legacy makes it a prime location for studying novel microbes due to its favorable characteristics, which support a wide range of biotechnological applications, including the synthesis of metallic nanoparticles. The number of published studies utilizing fungi from Brazilian habitats for producing metallic nanoparticles has not yet kept pace with this vast microbial richness, making it an underrepresented issue. This review aims to highlight the technological potential of fungus isolated from Brazilian habitats as a biocatalytic source to produce different metallic nanoparticles. A succinct bibliometric diagnosis based on the works in this review is also provided.



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Highlights

- Fungi (filamentous or yeast) isolated from the Brazilian biome.
- Great potential in different nanoparticle (NP) production.
- Ag NP stands out in the works described, followed by Ni, Cu, and other metals.

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- Conflict of interest
- Data availability statement
- Statement on the use of Artificial Intelligence tools
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1. Introduction

In 2001, first scientific papers about mycosynthesis of nanoparticles (NPs) was describe as novel technique in which fungi are used to synthesize nanoparticles (Mukherjee *et al.*, 2001a; 2001b). Over the next 20 years, living fungi and their extracellular metabolites were used to synthesize NPs of various chemical compositions, sizes, and shapes (Dhillon *et al.*, 2012; Khan *et al.*, 2018; Senapati *et al.*, 2014). These included metals such as Ag, Au, Cu, Pb, Pt, and bimetallic Ag-Au. Also synthesized were oxides such as BaTiO₃, Bi₂O₃, CoFe₂O₄, Co₃O₄, Fe₂O₃, Fe₃O₄, NiO, TiO₂, ZnO, and ZrO₂ (Priyadarshini *et al.*, 2021; Šebesta *et al.*, 2022).

The importance of metallic NPs is undeniable owing to their wide range of functionality and application potential. The synthesis of NPs can occur through two methods: the *Top Down* or *Bottom-Up* processes. In the *Top Down* process, NPs are produced by reducing their bulk form, as occurs in physical methods (Geonmonond *et al.*, 2018), and the *Bottom Up* methods involves ions and/or molecules, where the main reaction is oxy-reduction using chemical or biological methods (Borgschulte *et al.*, 2017).

Many chemical and physical processes exist for obtaining metal nanoparticles (Kumar *et al.*, 2018). Technological advances in the production and application of NPs have led to the development of reliable experimental protocols in the reproduction of properties (size, shape, load, and durability) and have become more sustainable (Ali *et al.*, 2021; Altammar, 2023). However, the physical-chemical routes, which are more widely employed, still require the use of toxic substances during certain stages of these processes (Dhillon *et al.*, 2012). Therefore, how to obtain metal nanoparticles using processing methods that are economically

viable and environmentally friendly remains the nanotechnological challenge (Li *et al.*, 2022).

The preparation of nanoparticles using biological methods is known to be more environmentally friendly. Green synthesis involves the production of NPs directly through the action of compounds from the process of cellular metabolism or co-factors and substances present in the enzymatic extract of microorganisms (Fadji *et al.*, 2022) (fungi, bacteria, yeast and actinomycetes) or plants (Dauthal and Mukhopadhyay, 2016; Konvičková *et al.*, 2018; Santos *et al.*, 2022).

Filamentous fungi have several advantages over plants, including key characteristics such as metal tolerance and the ability to bioaccumulate metals efficiently (Dhillon *et al.*, 2012). Filamentous fungi are easy to manipulate and manufacture in downstream processing (Pineda *et al.*, 2022) to form biomass and proteins that are susceptible to being used in other processes, which generates added value (Annamalai *et al.*, 2022). However, this process has its challenges owing to the long reaction time and the need to standardize the nanoparticles obtained, since the cellular metabolism succeeds to sensitive external sweeps (luminosity, pH, temperature, and air flow), which may affect the yield and shape of the nanoparticles (Mukherji *et al.*, 2018).

The nanoparticles production by biological processes is a form of microorganism defense itself which has resulted from a long evolutionary process. When under stress conditions, such as a high concentration of xenobiotic metals (Rai *et al.*, 2021), microorganisms eliminate toxic metals through various mechanisms. These include the accumulation of metal ions within cells, the active flow of metal ions through the cell membrane, and the chemical reduction of ions or bioaccumulation (Hulkoti and Taranath, 2014) (Fig. 1).

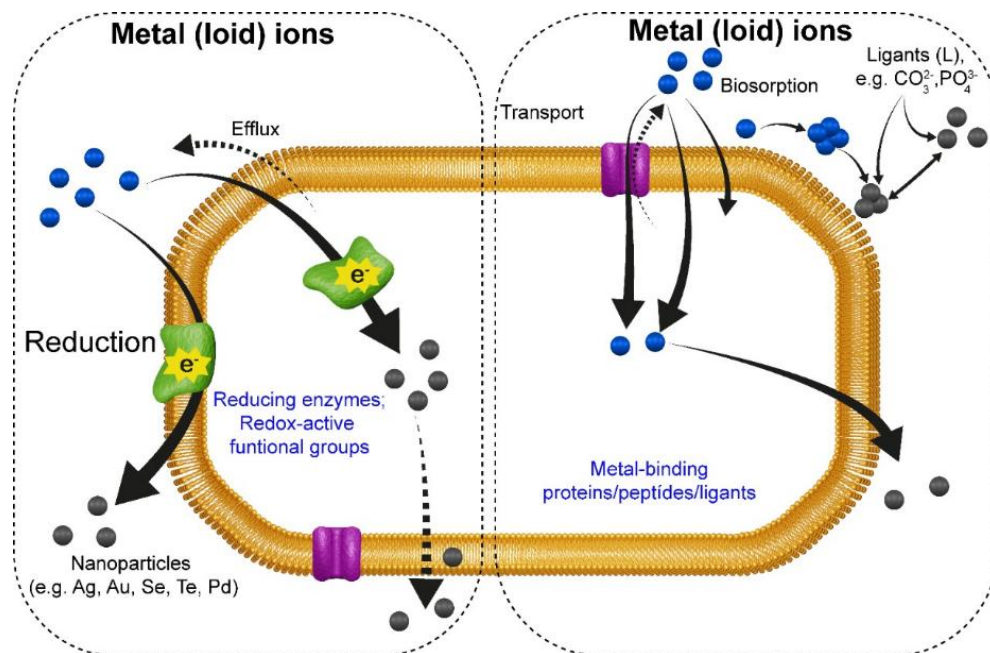


Figure 1. Some types of mechanisms that are involved in the formation of metal nanoparticles by cell fungi.

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Nanoparticle synthesis by fungi involves various mechanisms, which include the following: (1) Metal chelation and intra- and extracellular mineral formation fungi have the ability to produce large quantities of metal chelators, such as proteins of different types, polysaccharides, polypeptides, enzymes from sugar oxidoreductases or organic cofactors, which are substances that

bind to metals and reduce their harmful effects (Cánovas *et al.*, 2004; Katas *et al.*, 2018; Thapa *et al.*, 2017). These substances can function as surfactants and even as reducing agents or stabilizers throughout the biosynthesis process (Patra and Baek, 2014); (2) Biosorption this can occur through the cell wall of fungi, which consists mainly of polysaccharides such as chitin and β -1,3-glucan,

as well as proteins, polyphosphates, polypeptides, and other molecules (Šebesta *et al.*, 2022). Some filamentous fungi, such as *Aspergillus* sp. and *Penicillium* sp., have a higher sorption capacity than yeast and bacteria because their cell walls have a higher proportion of polysaccharides that contain many sorption sites, for example (Braydich-Stolle *et al.*, 2005). However, extracellular production enables size to be controlled with greater ease since NPs are precipitated outside of cells and are devoid of unnecessary cellular components, unlike intracellular ones, which tend to attach to the biomass cell wall and are difficult to purify (Slavin and Bach, 2022); (3) Bioaccumulation and compartmentation—this is one way that fungi can sequester the excess metal ions in specific cellular compartments, mainly vacuoles (Martínez *et al.*, 2022). The vacuoles act as sites for metal precipitation and NPs formation for various metals (Priyadarshini *et al.*, 2021); (4) Efflux Pump to prevent the harmful effects of excessive metal ions on their cellular functions, microorganisms have developed active efflux systems that pump out the metals from their cytoplasm (Coleman *et al.*, 2009; Musiol, 2023). These efflux systems are key to the metal resistance of many microbial species (Šebesta *et al.*, 2022). Active efflux is a type of transport mechanism that requires energy to move substances across the membrane against a concentration gradient (Hall, 2002).

Brazil's diverse biomes, such as the Amazon, Pantanal, Cerrado, Atlantic Forest, and Caatinga, along with its extensive coastline of approximately 3.5 million km², which includes ecosystems like corals, dunes, mangroves, lakes, estuaries, and swamps, make the country a hotspot for filamentous fungal diversity (Araújo *et al.*, 2022; Valencia and Chamberg, 2013). Lewinsohn and Prado (2005) estimated the total number of known fungi species (13,090–14,510) and those predicted to occur (150,300–263,900) in Brazil. However, the vast microbial biodiversity of Brazil is not adequately represented in published studies on fungi for metallic nanoparticle production, making this an underrepresented research area. Moreover, all this biodiversity is threatened by the advance of the Anthropocene, making the race for sustainable bioprospecting an urgent and indispensable issue.

Therefore, in this work we gathered data on the production of metallic nanoparticles produced by fungi isolated from Brazilian environments. It is noteworthy that most published studies involving these fungi have focused on the synthesis of silver nanoparticles, due to the applicability of the metal in the nanometric form. The most cited genera include *Aspergillus*, *Fusarium*, *Trichoderma* and *Rhodotorula*. Genera of low citation are *Scedosporium*, *Penicillium*, *Hypocrea*, and *Bionectria*. In this context, we conducted a bibliometric analysis to extract specific information from the articles used in this review. To accomplish this, we developed a search strategy using the titles of the articles. We then accessed the international scientific database Scopus to obtain the necessary information, following a methodology proposed by Ucella-Filho *et al.* (2022) and Lucas *et al.* (2023), with some adaptations. The data obtained were processed using the function “biblioshiny” in “bibliometrix” (Aria and Cuccurullo, 2017) of the software R Core Team (2021) and the VOSviewer version 1.6.15 (Eck and Waltman, 2010), software recommended for bibliometric analysis.

2. Genus included in this review

2.1. Genus *Aspergillus*

Aspergillus is a diverse genus of fungi that can produce several types of molecules through biological activity (Shah *et al.*, 2023; Wang *et al.*, 2021; Zhang *et al.*, 2018). These include

bioactive peptides/proteins, lectins, enzymes, hydrophobins and aegerolysins (Frisvad and Larsen, 2015). This common genus has been classified based on morphology many times (185) and currently contains over 200 species (Amaike and Keller, 2011). These species are widely used in biotechnology to produce organic acids, pharmaceuticals, proteins, and enzymes (Cleveland *et al.*, 2009; Meyer *et al.*, 2011) with applications in food, medicine, agriculture, and industries involving biotransformation processes (Birolli *et al.*, 2015; Salvadori *et al.*, 2014b).

Salvadori *et al.* (2014b) used the dead, dried, and live biomass of filamentous fungus *Aspergillus aculeatus*, isolated from the area of a copper mine in the Amazon region, for NiO nanoparticle bioproduction using the follow conditions: contact time of 90 min, pH 4.0, temperature of 30 °C, agitation speed of 150 rpm, and a solution containing 100 mg·L⁻¹ Ni (II). This study showed that the size of the nickel oxide nanoparticles was about 5.89 nm (Table 1, entry 1). Interest in this work was the comparison of dead biomass with dried and living biomass. The authors also demonstrated that dead biomass showed a higher sorption capacity of metal (19.6 mg·g⁻¹) compared with dry and living biomass (Salvadori *et al.*, 2014b). The mechanism of forming NiO nanoparticles using the dry biomass of *A. aculeatus* occurs preferentially through biosorption due to the presence of the acetamide group of chitins, amino and phosphate groups in nucleic acids, amino groups, starch, sulfhydryl and carboxy in proteins, and hydroxyls in polysaccharides. Their results demonstrated how biomass shape can affect catalytic activity (Mullen *et al.*, 1992; Salvadori *et al.*, 2015).

Aspergillus tubingensis, isolated as an endophytic fungus from *Rhizophora mangle*, was employed in the bioproduction of AgNPs of 35 nm (Table 1, entry 2) measured by Transmission Electron Microscopy (TEM) (Nano ZS Zetasizer, Malvern Instruments Corp, UK) analysis and with Zeta potential of +8.48 mV (Ballottin *et al.*, 2016). Fourier Transform Infrared (FT-IR) (ABB Bomen, MN series, USA) analysis enabled us to state that proteins adsorbed by the AgNPs did not suffer relevant secondary structure alteration upon their physical interaction with the AgNPs or when covalently bonded to them, thus reinforcing that the formation of AgNPs occurred through metal chelation. All materials were purchased from Sigma-Aldrich (St. Louis, MO, USA). In other work employing *A. tubingensis*, isolated from *Laguncularia racemosa* collected in a preserved mangrove forest located in Cananeia Coast in São Paulo State, Brazil, was reported for Rodrigues *et al.* (2013) to produce AgNPs of 10–35 nm (Table 1, entry 3). Nanoparticles were rapidly synthesized in the first 6 h from the absorbance increase at 440 nm, and which at 96 h reached the maximum absorbance; furthermore, the AgNPs showed pronounced antifungal activity against *Candida* sp., frequently occurring in hospital infections, with minimal inhibitory concentration in the range of 0.11–1.75 µg/mL. Exotic mediums such as Brazilian mangrove forests, where *A. tubingensis* was isolated, reveal a large reservoir of fungal genetic diversity and highlight the substantial differences between fungal communities associated with different plant tissues, plant species, impacted sites, and climatic seasons (Rodrigues *et al.*, 2013). However, little geoclimatic information has been noted about the isolation of the microorganism (Kandasamy and Kathirvel, 2023; Sebastianes *et al.*, 2013).

Gade *et al.* (2008) conducted one of the first studies on the formation of metallic nanoparticles mediated by fungal cells isolated from the Brazilian biome. In their study, cells from *Aspergillus niger* isolated from soil were used for the extracellular synthesis of silver nanoparticles with a diameter of around 20 nm (Table 1, entry 4). For the synthesis of nanoparticles, AgNO₃

(1 mmol·L⁻¹) was added to 50 mL of fungal filtrate, agitated at 2 h, 25 °C and 120 rpm. In addition, the maximum antibacterial activity of silver nanoparticles was observed when treated against *S. aureus*, whereas the minimum activity was recorded against *E. coli* (Gade *et al.*, 2008).

2.2. Genus *Fusarium*

Fusarium sp. is filamentous fungi belonging to the Ascomycota class. This genus was first described in 1809, with the main characteristic being the presence of canoe- or banana-shaped conidia (Pessôa *et al.*, 2017). *Fusarium* species are soil-borne vascular wilt pathogens, which are among the most important phytopathogenic and toxigenic fungi (Pessôa *et al.*, 2017). However, the metabolites *Fusarium* sp. products can be applied for biotechnology activities that have importance in the medical field (Nirmaledevi *et al.*, 2014).

Fusarium proliferatum (IOC 4682/IAL 7246), isolated from the Brazilian biodiversity via epiphytes from the leaf of the mangrove plant *Rhizophora mangle* and deposited at the microorganism collection from Instituto Oswaldo Cruz (IOC, Rio de Janeiro, RJ, Brazil), demonstrated the ability to synthesize silver nanoparticles measuring 59 nm, PDI 0.199, and -15 mV of Zeta potential (Table 1, entry 5) at 28 °C and 150 rpm for 72 h with a pH 6. AgNPs produced by *Fusarium proliferatum* cells (IOC 4682/IAL 7246) were evaluated against the phytopathogenic species of *Fusarium*, such as *F. sacchari*, *F. subglutinans*, *F. oxysporum* (WLA-FP07), *F. oxysporum* (WLA-FP25), and *F. verticillioides*. The AgNPs showed the same values for MICs and fungicide concentration (FC), indicating that the activity of AgNPs is more fungicidal than fungistatic (Ribeiro *et al.*, 2023).

In the second example, *Fusarium oxysporum* obtained from the Laboratory of Ceramic and Composite Materials (CERMAT/UFSC) was used to produce AgNPs. Using standard biosynthesis parameters (10⁻³ mol L⁻¹ AgNO₃; 10 g biomass; 28 °C; and 5-day growth time), nanoparticles were produced up to 30 nm with -33 mV (Table 1, entry 6). The authors studied the influence of the concentration of the metal in the fungal medium and concluded that the average particle size increases as the concentration of silver in the medium increases (0.01 to 0.1 mol L⁻¹) (Almeida *et al.*, 2017). This tendency is expected because successful metal reduction is associated with an excess of enzymes or biomolecules available in the biosystem. However, many of these studies do not show the relationship between enzymatic potential and the quantitative data of the nanoparticle, which would shed more light on the involved mechanisms and thus improve the methods for standardizing nanoparticles.

Durán *et al.* (2005) used *Fusarium oxysporum* from ESALQ-USP Genetic and Molecular Biology Laboratory-Piracicaba, S.P., Brazil, to produce AgNPs measuring 20–50 nm (Table 1, entry 11). Briefly, approximately 10 g of *F. oxysporum* biomass was used in a conical flask containing 100 mL of distilled water and AgNO₃ solution (10⁻³ mol·L⁻¹).

Whole cells of *Fusarium oxysporum* used in the production AgNPs were reported by Ishida *et al.* (2013). The *F. oxysporum* 07 SD strain was provided by the Center for Environmental Sciences, University of Mogi das Cruzes, Mogi das Cruzes, state of São Paulo, Brazil. It was observed that at AgNO₃ concentrations of up to 1.0 mmol·L⁻¹ to 60 days at room temperature in the dark was the most efficient condition for producing AgNPs measuring 3.4–26.8 nm (Table 1, entry 12). This effect was attributed to the lack of functional groups available for the reaction when the metal precursor concentration was increased. In addition, this study showed an elevated level of antifungal activity against *Candida* and

Cryptococcus, with minimum inhibitory concentration values ≤ 1.68 µg mL⁻¹ for both genera. Morphological alterations of *Cryptococcus neoformans* treated with these AgNPs were observed, such as disruption of the cell wall and cytoplasmic membrane and the loss of the cytoplasmic content.

2.3. Genus *Rhodotorula*

Rhodotorula is an anamorphic yeast genus belonging to the phylum Basidiomycota (Perniola *et al.*, 2006). These are the common pigmented environmental inhabitants that produce carotenoid pigment (Akhtyamova and Sattarova, 2013) that can be isolated from environmental sources such as soil, fresh water, and air samples as well as milk, salad, and fruit juice in addition to shower curtains and toothbrushes (Tournas *et al.*, 2006a; 2006b).

Rhodotorula glutinis and *Rhodotorula mucilaginosa*, isolated from the soil around the Pici Campus, Federal University of the Ceará (Fortaleza, Ceará, Brazil); Latitude: 3°44'20.832" S, Longitude: 38°34'12.483" W; and 3°44'21.750" S, 38°34'12.350" W, respectively, were reported by Cunha *et al.* (2018) to obtain AgNPs. These silver nanoparticles were characterized by UV-Vis (Thermo Scientific GENESYS™ 10 s, Fortaleza, Ceara-Brazil), FTIR (Perkin-Elmer FT-IR, Fortaleza, Ceara-Brazil), TEM (Advanced Microscopy Techniques, Danvers, MA, USA), DLS (Dynamic Light Scattering) (Zetasizer Nano ZS, (Malvern Instruments in United Kingdom), and AFM (Atomic Force Microscopy) (a Multimode Nanoscope IIIa (Bruker, CA, USA), had a size of 15.45 nm (Table 1, entry 13) and 13.70 nm (Table 1, entry 14) when analyzed by TEM (Advanced Microscopy Techniques, Danvers, MA, USA). Additionally, AgNPs were shown to possess antifungal activity alone, and when associated with fluconazole, against *C. parapsilosis*. Many AgNPs may serve as an option for treating fungal infections. All materials were obtained from Himedia-India, Dinâmica-São Paulo-Brazil, and Sigma-USA).

Agressott *et al.* (2020) obtained silver nanoparticles through biological synthesis using fungi of the species *Rhodotorula glutinis* and *Rhodotorula mucilaginosa* found in ordinary soil on the campus of the Federal University of Ceará (Brazil). This laboratory synthesis process represents a green approach to the production of this nanomaterial and requires a few days to prepare the culture medium for the biosynthesis of the NPs. The nanoparticles were analyzed by TEM, which characterized the AgNPs obtained from *Rhodotorula glutinis* and *R. mucilaginosa* with average sizes of 58 nm (Table 1, entry 15) and 30 nm (Table 1, entry 16), respectively.

Rhodotorula mucilaginosa, isolated from the water collected from a pound of copper waste from Sossego mine, located in Canã dos Carajás, Pará, Brazil, was used by Salvadori *et al.* (2014c) in the bioprocess for the synthesis of metallic copper under differing conditions of pH (2–6), temperature (20–60 °C), contact time (5–360 min), CuCl₂·2H₂O concentration (25–600 mg·L⁻¹), and agitation rate (50–250 rpm). The nanoparticle was spherical, with an average size of 10.5 nm (Table 1, entry 17), confirmed by X-ray photoelectron spectroscopy analysis. In this work, the authors showed the high concentration of intracellular copper NPs in the yeast cell, which was uniformly distributed (monodispersed) without significant agglomeration in the cytoplasm and absent in control.

2.4. Genus *Trichoderma*

Trichoderma is a genus of filamentous fungi that displays a range of lifestyles and interactions with other fungi, animals, and

plants (Druzhinina *et al.*, 2011) and different water-related habitats including marine environments and drinking water (Kredics *et al.*, 2014). *Trichoderma* spp. are characterized by rapid growth, mostly bright green conidia, and a repetitively branched conidiophore structure (Druzhinina *et al.*, 2011). Furthermore, *Trichoderma* is found to be an effective, beneficial biological agent, with active ingredients in over 200 agricultural products such as biopesticides, biofertilizers, bio-growth enhancers, and bio-stimulants that are marketed across the globe (Alghuthaymi *et al.*, 2022).

T. harzianum was also used to obtain silver nanoparticles. Guilger-Casagrande *et al.* (2017) demonstrated the biogenic synthesis of silver nanoparticles of 20–30 nm using *T. harzianum* contained in the product Ecotrich™ (Estrada Municipal Carlos Gebim, 2353 - Laranja Azeda. Bom Jesus dos Perdões, São Paulo, Brazil) (Table 1, entry 18) as the reducing agent. The authors found that the nanoparticles inhibited the germination of resistance structures in the pathogen *Sclerotinia sclerotiorum*. Another important finding described by the authors was the effect that nanoparticles had on the soil. There was evidence of some partial effects on bacteria, with the possible recovery of the microbiota over time, a crucial step in controlling pathogens without negatively affecting the soybean germination process.

Another work using the isolation of fungi within the Brazilian environment was that of Bilesky-José *et al.* (2021) in the green synthesis of biogenic iron oxide nanoparticles using *Trichoderma harzianum* as a stabilizing agent. To date, this is the only study that has described the formation of metallic nanoparticles by fungi isolated from a Brazilian environment, which reinforces the need for greater applications of these microorganisms in different metals. The results indicated that nanoparticles consisting of hematite ($\alpha\text{-Fe}_2\text{O}_3$) had a mean size of 207 nm in diameter, a polydispersity index of 0.45, and a Zeta potential of 13 mV (Table 1, entry 19). However, XRD analysis (Shimadzu XRD-6000, Sorocaba, São Paulo, Brazil) revealed that although the crystallographic peaks described confirmed the synthesis of hematite from FeCl_3 , other typical signs of the hematite diffractogram were absent (such as at 35.7, 49.5, 62.6, 64 and 72.3°). It is possible that the sample was not a pure iron oxide crystal and that the signals were suppressed by the presence of other signals. A third example of the use of *Trichoderma harzianum* cells was in the production of AgNP. The authors report the biosynthesis of AgNPs using the filtrates of *T. harzianum* cultured in the presence (AgNP-TS) and absence (AgNP-T) of the cell wall of *Sclerotinia sclerotiorum*. The AgNP-TS and AgNP-T presented mean hydrodynamic diameters of 57 and 81 nm, respectively (Table 1, entry 20). The values of polydispersity and Zeta potential were 0.49 and -18 mV for AgNP-TS and 0.52 and -18 mV for AgNP-T, respectively. (Table 1, entry 21). The authors also determined the enzymatic activity (β -1,3-glucanase, NAGase, chitinase, and acid protease) present in the filtrates, reinforcing the mechanism of action via metal chelation. This study opened the possibility for future explorations of biogenic nanoparticles from *Trichoderma harzianum*, with the potential to control *S. sclerotiorum* and other agricultural pests (Guilger-Casagrande *et al.*, 2019).

Salvadori *et al.* (2014a) showed interesting results using in the copper nanoparticles produced by dead biomass of *Trichoderma koningiopsis* with an average size of 87.5 nm (Table 1, entry 20) exhibited a spherical shape. According to the authors, the presence of proteins as stabilizing agents of the nanoparticles and uptake of copper nanoparticles using dead fungal biomass represent a low-cost green process and bioremediation-impacted location. The *T. koningiopsis* was isolated from the sediment collected in a copper waste pond at the Sossego mine, which belongs to the company Vale S.A. located in Canaã of the Carajás, Brazil.

Different *Trichoderma* spp. isolated from the *Bertholletia excelsa* (Brazil-nut) seeds and the soil were used by Ramos *et al.* (2020) to biosynthesize AgNPs and showed a directly proportional relationship between decreased pH and decreased hydrodynamic diameters of particle size (Fig. 2a, as the growth of *Trichoderma* sp. 01 in basic medium (pH 8) resulted in the formation of particles measuring 260 nm, whereas in pH 5 resulted in particles of 150 nm (Table 1, entry 21). These AgNPs showed potential against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus* and *E. faecalis*) active bacteria.

2.5. Different genera

The epiphytic fungus *Bionectria ochroleuca*, isolated from *L. racemose* collected in a preserved mangrove forest located in Cananea Coast in São Paulo State, Brazil, was reported by Rodrigues *et al.* (2013) for AgNPs biosynthesis measuring 35 ± 10 nm in the first 6 h of the reaction (Table 1, entry 22). The authors observed the presence of S and N around the AgNPs, which indicated the presence of proteins around the nanoparticles. Their study was the first to observe isolated fungi from Brazilian mangrove to obtain AgNP for application in antifungal activity against *C. albicans* and *Thichophyton mentagrophytes* with MIC₈₀ values between 5.88 to 41.18 μM . These nanoparticles (in low concentrations) showed inhibited fungal growth to 90% of that of the traditional chemical production of AgNPs as well as huge stability up to 90 days due to protein capping that stabilized the AgNPs.

Fungal filtrate from *Duddingtonia flagrans* was used by Barbosa *et al.* (2019) to produce AgNPs of 3.25 nm (Table 1, entry 23). This isolated fungal filtrate comes from Brazilian soil located in Viçosa, Minas Gerais. The filtrate was prepared from fungal isolate culture in 200 mL of nutrient medium (peptone, yeast extract, potassium phosphate, and lactic acid), in orbital agitation 120 rpm, 25 °C for 10 days. One hundred milliliters of 1 mmol·L⁻¹ AgNO₃ solution was added to the fungal filtrate at a concentration of 1:50, and the new solution was kept in an agitator at 120 rpm, 60 °C, in the dark and AgNO₃ solution (1 mmol·L⁻¹) was then added. The AgNPs from fungal filtrate *D. flagrans* exhibited nematocidal activity, being the only ones capable of penetrating the cuticle of the larvae, which caused changes in the tegument, and consequently, the death of the nematode.

Sharma *et al.* (2021) obtained NiONPs using dead fungal biomass from *Hypocrealixii* measuring 1.25–3.8 nm (Table 1, entry 24). *Hypocrealixii* was isolated from the pondwater of copper waste from a Sossego mine, located in Canaã of the Carajás, Pará, in the Brazilian Amazonia region. The fungal biomass was prepared on Sabouraud Dextrose Agar. The recipient was put in orbital agitation 20–250 rpm, 20–60 °C for 95–300 min and nickel concentration at 50–500 mg·L⁻¹ in pH range of 2–6. The optimal conditions research to NiO NPs was pH 4, 30 °C, 150 rpm, 1 g of fungal biomass, and 100 mg·L⁻¹ of NiO for 90 min. The authors demonstrated that dead fungal biomass was excellent for the synthesis of the nickel oxide NPs in metal aqueous solution. The key role of dead fungal biomass is as a reduction agent to allow the oxidation of metallic Ni to nickel oxide and as a stabilizing agent. This method of obtaining NPs from dead fungal biomass is highly relevant for the sustainable process of nanoparticle synthesis of Nickel and other metals that permits decontamination of aqueous metal media (Salvadori *et al.*, 2015).

This microorganism also was used by Salvadori *et al.* (2013) in the CuNP biosynthesis with a size of 24.5 nm (Table 1, entry 25) from dead fungal biomass. This research shows that the percentage of removal of copper by dead biomass was higher compared to that

observed for live and dried biomass, and that the CuNPs obtained were in the cell wall, but not in the cytoplasm or cytoplasmic membrane. These CuNPs show a high copper biosorption capacity of 19.0 mg/g in 60 min, pH 5, 40 °C at 150 rpm. The authors conclude that the use of dead fungal biomass can be a suitable alternative as the method is green and cheap as well as easy to carry out and technically feasible for the bioremediation of wastewater fonts. They also recommend CuNP biosynthesis as a suitable method for the large-scale production of metal-nanoparticles.

Copper nanoparticle biosynthesis has also been achieved using endophytic fungus *Phaeoacremonium* sp. isolated from *Bertholletia excelsa* (Brazil nut). Morais *et al.* (2021) obtained Copper nanoparticles of up to 66.84 nm (Table 1, entry 26) with a Zeta potential of -34 mV. The authors show the use of the Amazon-derived fungus *Phaeoacremonium* sp for the synthesis of CuNPs can be achieved under green chemistry conditions at a low cost and for metal decontamination. Thus, this method can make possible the use of other new Amazon-derived fungi to obtain nanoparticles from copper and other metals (Fig. 2b).

Table 1. Nanoparticles produced for different fungal species from the Brazilian biome.

Genus <i>Aspergillus</i>					
1	Amazon	<i>A. aculeatus</i>	NiONP	5.89	Salvadori <i>et al.</i> (2014b)
2	Atlantic forest	<i>A. tubingensis</i>	AgNP	35	Ballottin <i>et al.</i> (2016)
3	Atlantic forest	<i>A. tubingensis</i>	AgNP	10–35	Rodrigues <i>et al.</i> (2013)
4	Atlantic forest	<i>A. niger</i>	AgNP	20	Gade <i>et al.</i> (2008)
Genus <i>Fusarium</i>					
5	Mangrove forest	<i>F. proliferatum</i>	AgNP	43–120	Gaikwad <i>et al.</i> (2013)
6	No information	<i>F. oxysporum</i>	AgNP	30	Gaikwad <i>et al.</i> (2013)
11	Atlantic forest	<i>F. oxysporum</i>	AgNP	20–50	Durán <i>et al.</i> (2005)
12	Atlantic forest	<i>F. oxysporum</i>	AgNP	3.4–26.8	Ishida <i>et al.</i> (2013)
Genus <i>Rhodotorula</i>					
13	Caatinga	<i>R. glutinis</i>	AgNP	15.45	Cunha <i>et al.</i> (2018)
14	Caatinga	<i>R. mucilaginosa</i>	AgNP	13.70	Cunha <i>et al.</i> (2018)
15	Caatinga	<i>R. glutinis</i>	AgNP	58	Agressott <i>et al.</i> (2020)
16	Caatinga	<i>R. mucilaginosa</i>	AgNP	30	Cunha <i>et al.</i> (2018)
17	Amazon	<i>R. mucilaginosa</i>	CuNP	10.5	Salvadori <i>et al.</i> (2014c)
Genus <i>Trichoderma</i>					
18	No information	<i>T. harzianum</i>	AgNP	20–30	Guilger-Casagrande <i>et al.</i> (2017)
19	No information	<i>T. harzianum</i>	FeNP	207	Bilesky-José <i>et al.</i> (2021)
20	Amazon	<i>T. koningiopsis</i>	CuNP	87.5	Salvadori <i>et al.</i> (2014a)
21	Amazon	<i>Trichoderma</i> sp.	AgNP	150–260	Ramos <i>et al.</i> (2020)
Different genera					
22	Atlantic forest	<i>Bionectriaochroleuca</i>	AgNP	10–35	Rodrigues <i>et al.</i> (2013)
23	Atlantic forest	<i>Duddingtoniaflagrans</i>	AgNP	3.25	Barbosa <i>et al.</i> (2019)
24	Amazon	<i>Hypocrealixii</i>	NiONP	1.25–3.8	Sharma <i>et al.</i> (2021)
25	Amazon	<i>Hypocrealixii</i>	CuNP	24.5	Salvadori <i>et al.</i> (2013)
26	Amazon	<i>Phaeoacremonium</i> sp.	CuNP	66.84	Morais <i>et al.</i> (2021)

Source: Elaborated by the authors.

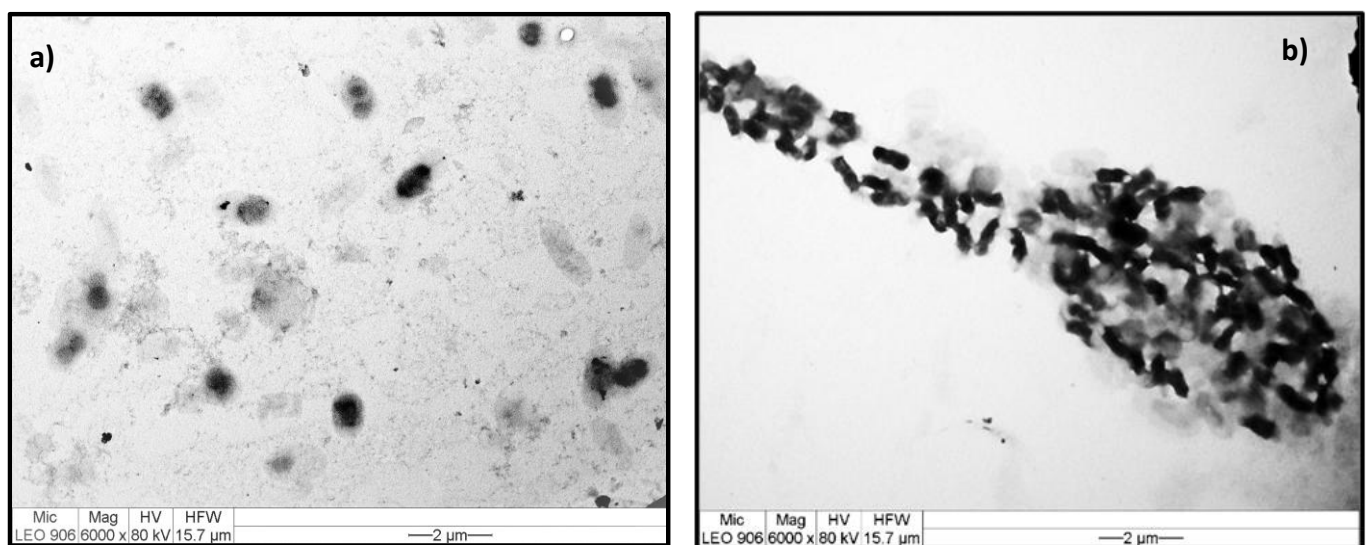


Figure 2. Transmission Electron Microscopy (TEM) images of silver nanoparticles (AgNPs) (a) synthesized by *Trichoderma* sp. and (b) copper nanoparticles (CuNP) from *Phaeoacremonium* sp.

Source: Adapted from Ramos *et al.* (2020) and Morais *et al.* (2021) (Copyright 2020, with permission from Springer Nature).

3. Bibliometric analysis

Through the bibliometric data, it was observed that, except for the Midwest region, the other Brazilian regions presented corresponding research groups. However, the Southeast region of Brazil concentrates most of the published research on the use of fungi isolated from Brazilian environments to obtain metallic nanoparticles, corresponding to 89% of the total of these studies. Among the states of this region, São Paulo and Rio de Janeiro stood out with 68 and 11%, respectively (Fig. 3). These results are expected, since São Paulo is the state with the largest number of notable research centers (public and private), such as the University of São Paulo (USP) and the State University of Campinas (UNICAMP) among others. Consequently, there are a greater number of research groups related to the area of biotechnology, chemistry, physics, biology, and related areas that study the use of microorganisms in the production and application of metallic nanoparticles. In addition, São Paulo Research Foundation (FAPESP) is responsible for the largest volume of resources made available for research at the state agency level. The

concentration of studies in this region can be attributed to factors such as: (i) an infrastructure that is favorable to the development of research; (ii) a high concentration of researchers due to the presence of several research centers, which drives the advancement of research and highlights this region as an important research center in the country; and (iii) industrial demand and application, because the Southeast region is home to a large number of industries, including sectors such as petrochemicals, fine chemicals, and food, among others; (iv) consolidated research groups (Monteiro Neto *et al.*, 2015), with this, the industrial sector requires, and in some cases also finances, innovative and efficient solutions in the area of biotechnology, which stimulates the realization of this line of research.

However, this unevenness in the involvement of research groups in the production of metallic nanoparticles by fungi (filamentous or yeast) isolated from Brazilian environments is worrisome from the point of view of the still unexplored Brazilian fungal biodiversity given that, as previously noted, Lewinsohn and Prado (2005) estimated the total number of known fungi species to be 13.090–14.510 and predicted 150.300–263.900 to occur in Brazil.

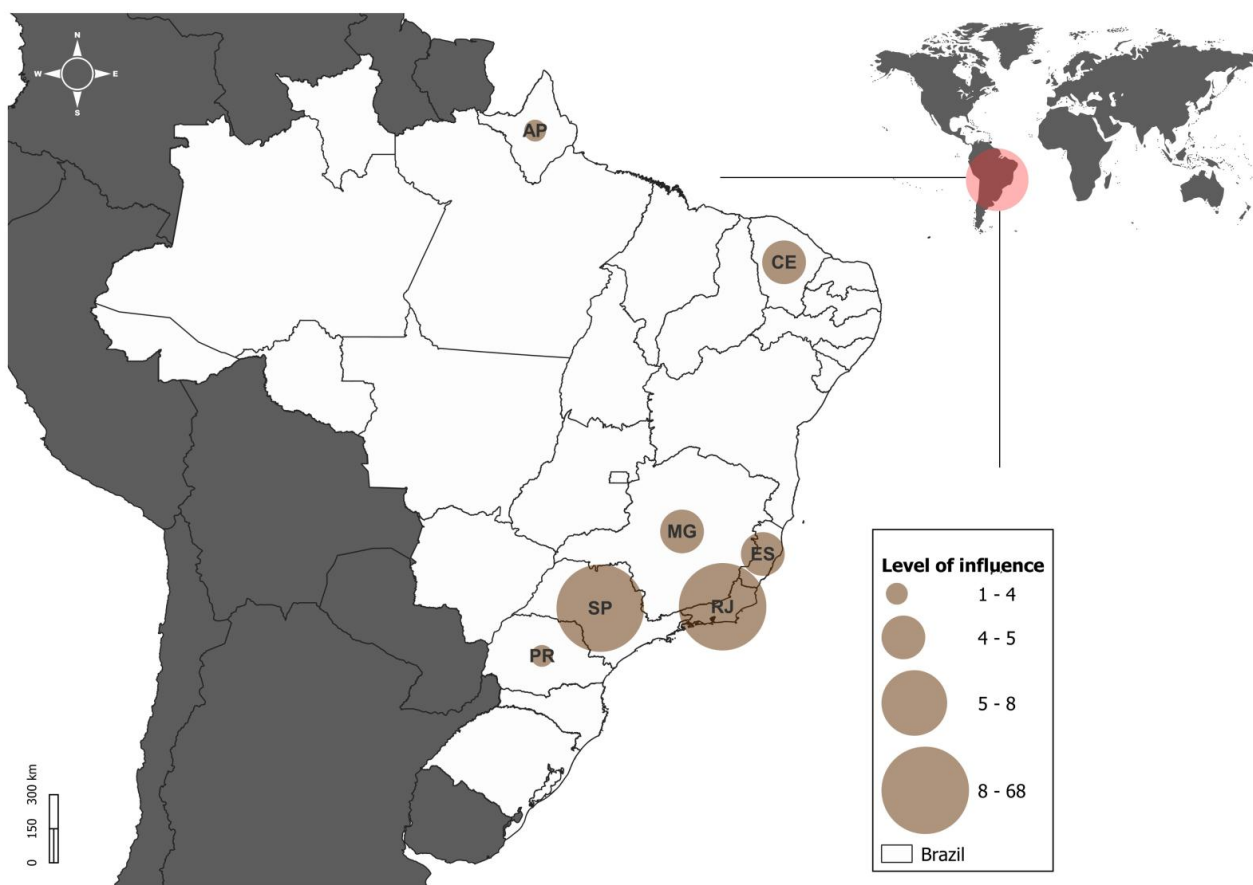


Figure 3. Contribution of the states of Brazil to research on metal nanoparticles.

Source: Elaborated by the authors.

The bibliometric data also identified a collaboration of Brazilian groups with those of other countries. The most frequent partnerships occurred between Brazil and Chile, totaling two collaborations, followed by Brazil and Germany ($n = 1$) and Brazil and Ireland ($n = 1$) (Fig. 4). These data highlight the low level of relationship of Brazilian research groups with other research groups abroad even though in recent years Brazilian research groups have overseen studying the production of metallic

nanoparticles by filamentous fungi. International cooperation strengthens relations between researchers and institutions, boosting high-impact scientific production and the collaborative solution of global challenges (Dusdal and Powell, 2021; Franco and Pinho, 2019). Therefore, when it comes to the production of metallic nanoparticles by filamentous fungi, overcoming this challenge can be considered one of the main bottlenecks in the scientific field.

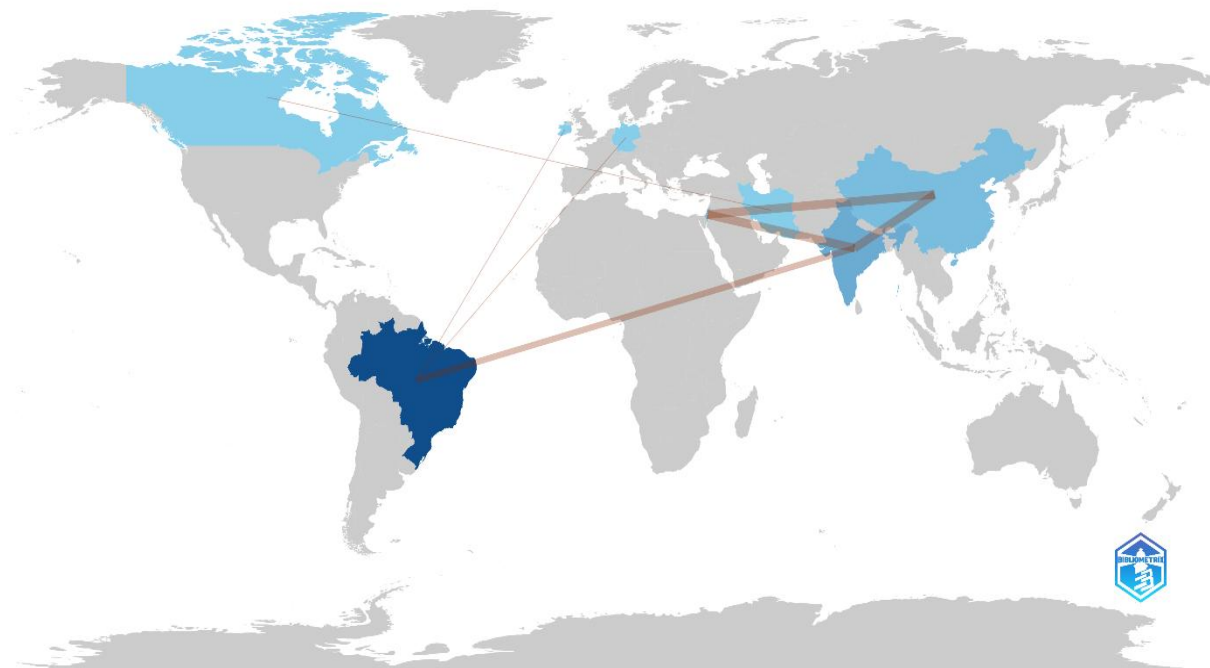


Figure 4. Collaboration network between countries.

Source: Elaborated by the authors.

According to the data collected, the articles are concentrated on the areas of Immunology and Microbiology ($n = 9$), Biochemistry, Genetics and Molecular Biology ($n = 8$) and Chemical Engineering ($n = 6$) (Fig. 5). This distribution reflects the multidisciplinary nature of the studies, where the same article can be indexed in more than one area. The predominance in these three areas can evidently be attributed to the relationship of fungi by microbiology, justifying the indexation in the areas of Immunology and Microbiology and Biochemistry, Genetics and Molecular Biology, well as (bio)catalysis as chemical procedure,

which justifies the presence in Chemical Engineering. The multidisciplinary approach is essential in these studies of biocatalysts from filamentous fungi and allows the integration of biology, chemistry, and engineering knowledge to explore the full potential of these microorganisms as biocatalysts. This, in turn, enables a comprehensive understanding of the mechanisms involved in the biosynthesis of metallic nanoparticles and facilitates the development of innovative and efficient solutions in this area of research.

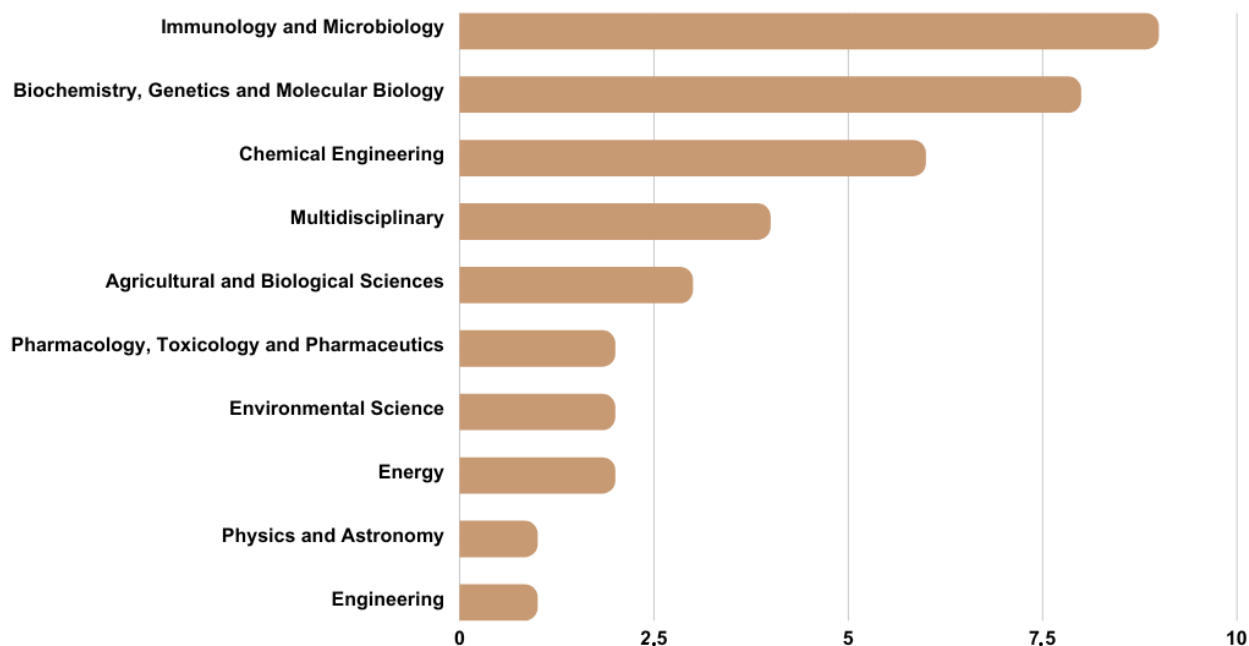


Figure 5. Main indexing areas of the articles.

Source: Elaborated by the authors.

We identified and quantified 49 keywords defined by the authors cited in the articles listed in the production of metallic nanoparticles from fungi (filamentous or yeast) isolated from Brazilian environments. The most frequent terms were “Silver nanoparticles” ($n = 4$), “biosynthesis” ($n = 2$) and “*Aspergillus tubingensis*” ($n = 2$), presenting, respectively 15, 8, and 6 connections to other keywords (Fig. 6). According to Ucella-Filho *et al.* (2022), the interaction network is represented considering the frequency of each keyword, evidenced by the width of the areas of

the circles. Terms that occur more frequently are closer to each other, while links (lines) indicate the interaction between the terms. The thicker the links, the more often the terms are connected. The clusters represent the grouping of items, presented in distinct colors on the map. The analysis of the keywords reveals the focus of interest of the studies, and thus these results indicate the interest of researchers in the development of research involving metal nanoparticles and the use of *Aspergillus tubingensis* as biocatalysts.

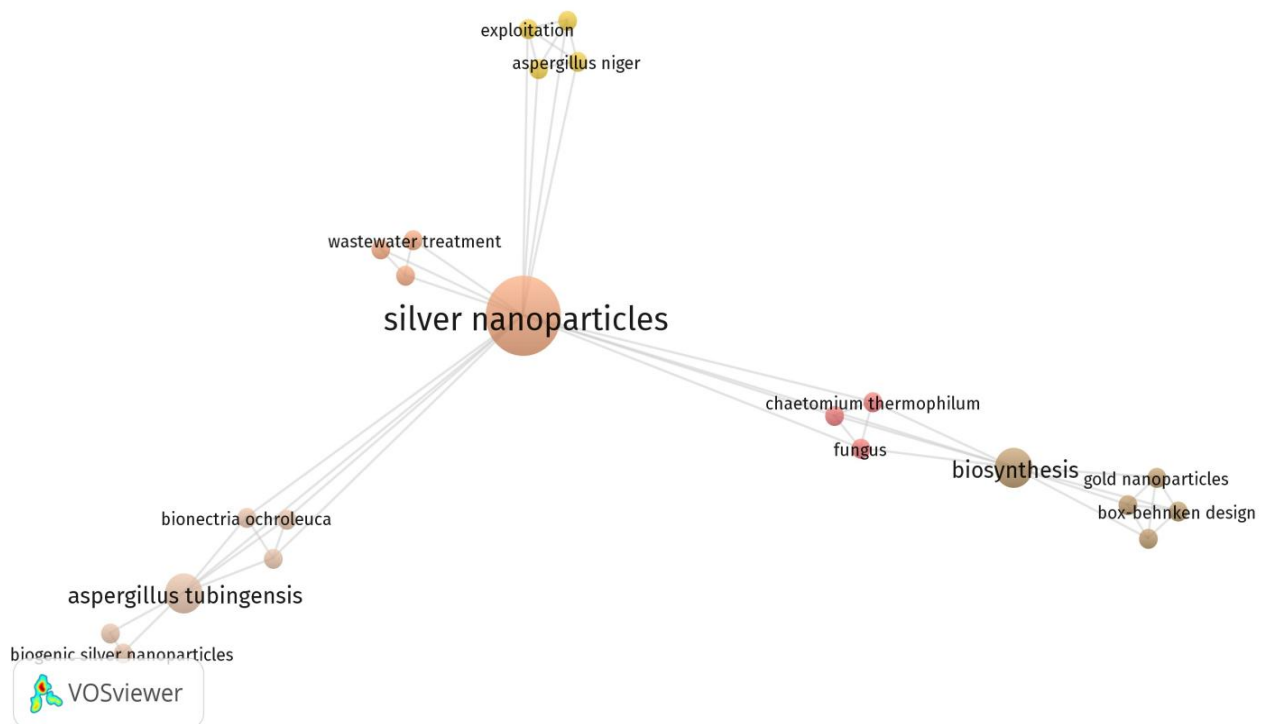


Figure 6. Network of interactions between author keywords. Total number of terms in the network = 22. Minimum number of occurrences of a term = 1. Total Clusters formed = 4.

Source: Elaborated by the authors.

4. Conclusions

Fungi (filamentous or yeast) isolated from Brazilian biome have shown immense potential in different nanoparticle production as well as in various fields, including medicine and environmental biotechnology. Despite the advantages biological systems may provide as a sustainable, resource-efficient and an economical method of synthesis, compared to conventional physical-chemical methods, Brazil has thus far presented a low number of works involving extracts or total cells of fungi in the production of metallic nanoparticles. This is despite the vast potential for the bioprospection of fungi through the different biomes. Also observed in this review is that the Ag nanoparticle stands out in the works described. However other metals, such as Ni, and Cu were also involved in the biological system by fungi isolated from Brazilian biomes, and soon other elements are likely to be added to this list in view of the large numbers of nanoparticle-forming fungal species and susceptible elements, compounds, and minerals. Furthermore, based on the bibliometric analysis of the articles used in this review, three important points were identified to be considered in the development of new studies: (i) the expansion of research on the use of fungi as biocatalysts for the biosynthesis of metallic nanoparticles in different states and institutions in Brazil; (ii) the responsible exploitation of Brazilian

biodiversity in search of new species of fungi with biocatalytic potential; and (iii) the promotion of international cooperation between research groups from Brazil and other countries, with the aim of advancing research in this field. These actions have the potential to boost Brazil and make it a crucial source of research in this scientific niche.

Authors' contribution

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Conflict of interest

The authors declare that there is no conflict of interest.

Data availability statement

All datasets generated or analyzed during the current study are available from the corresponding author upon reasonable request.

Artificial Intelligence usage statement

The authors declare that there were no used artificial intelligence tools.

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