applied microbiology

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Capacity of the Fungi Trichoderma Koningiopsis and Talaromyces Verruculosus for Hg Leaching, Immobilization and Absorption During the Dissolution of Cinnabar

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Abstract: The present study investigates the potential capacity of fungi for the use in mercury (Hg) leaching and immobilization during the dissolution of cinnabar ore, the updated understanding of the mechanisms involved, and the evaluation of Hg absorption by these fungal strains. Two fungal strains are isolated from Hg-polluted soils in French Guiana and identified as *Trichoderma koningiopsis* and *Talaromyces verruculosus*. These fungal strains possess a high capacity for Hg resistance. The Hg concentrations causing 50% growth reduction (EC_{50}) are 5.9 and 1.5 ppm for *T. koningiopsis* and *T. verruculosus*, respectively. The results of medium-culture-containing cinnabar ore show that these fungal strains remove over 99% of the Hg content in the culture media by the end of the experiment. Fungal biomass decreases with increasing mercury concentration. The production of organic acids by fungi is observed for both fungal strains, leading to an acidic pH in the medium culture. Oxalic and citric acids are preferentially produced to dissolve Fe from minerals, which may impact Hg leaching. The results of this study provide evidence that the two fungi seem to have potential use for the bioremediation of Hg during the dissolution of cinnabar ores through biosorption mechanisms.

Keywords: bioabsorption; biodissolution; fungi; mercury; organic acids

Academic Editor: Sabina Fijan

Received: 5 November 2024
Revised: 10 January 2025
Accepted: 14 January 2025
Published: 27 January 2025

Citation: Balland, C.; Alphonse, V.; Jusselme, M.D.; Abbad-Andaloussi, S.; Bousserrhine, N. Capacity of the Fungi Trichoderma Koningiopsis and Talaromyces Verruculosus for Hg Leaching, Immobilization and Absorption During the Dissolution of Cinnabar. *Appl. Microbiol.* **2025**, *5*, 12. https://doi.org/10.3390/applmicrobiol5010012

1. Introduction

Mercury (Hg) is one of the major contaminants that may cause serious problems in human health and environmental damage due to its toxicity and the relatively uncomplicated uptake of Hg organic compounds produced by microorganisms [1,2]. The presence of naturally high background Hg levels in soil as well as that produced by anthropogenic activity, mainly illegal goldmining, has led to high Hg concentrations in French Guiana soils [3,4]. In French Guiana, the tropical weather, which is hot and humid throughout the year, exacerbates the natural phenomena of Hg-rich rock weathering that corresponds to the deterioration of minerals within them through contact with water and biological organisms and leads to the transport of elements by water. Due to the affinity of inorganic mercury (Hg(II)), the majority of Hg forms bonds with iron and alumina and is also absorbed by clay minerals [5]. Mercury pollution in French Guiana is due to illegal gold mining [4], which has led to the high Hg concentration in soils of up to 9 ppm, with 0.5 ppm in Ferralsols and 0.35 ppm in Gleysols [6]. These soils are an important environment where mercury can be transformed into various potentially toxic chemical forms. These toxic forms will subsequently be either more or less available and mobile in the soil, water, and atmosphere than before transformation. The spatial distribution of mercury in soils is linked not only

to the quantity and quality of the organic material, as well as those of iron and aluminium (oxy/hydr)oxides, but also to the biological activity in the soil [6].

The impact of mercury contamination on the soil functioning and microbial community has been studied for several years. Mercury is well known to be toxic to soil microorganisms and can reduce the diversity and activity of the soil microbial community. Many studies [6–9] have shown that the presence of mercury in soils results in a low density of microorganisms as well as a change in the microbial community structure according to their mobility and bioavailability [10,11]. Moreover, the impact of mercury on microorganisms depends on soil physicochemical parameters such as pH, redox potential, and dissolved organic carbon, which can modify the chemical form of mercury [12–14]. Bacterial responses to Hg-pollution stress by the transformation of Hg are well known [15–18]. As a result, Hg-resistant bacteria play an important role in the remediation of environmental Hg pollution [19,20].

In addition to bacteria, mushrooms (macro-fungi) are known for their ability to bioremediate Hg [21]. Fungi are a large part of the soil biomass and are known for their role in soil formation and the cycling of elements. Fungi play an important role in woody decomposition and the recycling of organic matter as well as in plant nutrition via mineral weathering in soils [22–24]. Many reports have emphasized the role of mushrooms (macrofungi) in Hg bioremediation due to their capacity to adsorb and accumulate Hg in their cell and/or fruiting bodies [25–27]. Moreover, many studies have shown that the inoculation of fungi, while in symbiosis with the plants, affects the accumulation of certain trace metals, such as copper and cadmium, and allows for the increase in the tolerance of host plants to these metals [21,28–30]. Thus, soil contamination with trace metals can not only change the balance of the soil at the level of biodiversity but also at the level of their function within organisms [31,32]. Although trace metal contamination is very toxic to some organisms, fungal species have the ability to survive and grow in polluted soils due to their ability to exude different chemical compounds, particularly organic acids [33,34]. Some fungi tolerate trace metals, allowing for their storage to reduce the risk to surrounding organisms, including transfer to plants, by producing exopolymers that trap the trace metals [35]. Several studies showed the high tolerance of *Aspergillus niger* to the presence of lead and chromium [7]. The absorption of trace metals, particularly mercury, depends on various factors, including soil physicochemical parameters, fungal species behaviour, and the temperature and climate of the region. In recent years, many studies have focused on the bioaccumulation of trace metals by fungi [9,21,36,37].

However, few studies have been conducted on the role of fungi (micro-fungi) in mercury dynamics (mobility and bioavailability) in soils and their capacity for Hg leaching and absorption, and the mechanisms involved are still poorly understood [38]. This work could lead to more sustainable alternatives for the remediation of mercury-contaminated soil through the use of fungi.

This study aims at evaluating the fungi potential to leach Hg, to absorb and identifying the various mechanisms involved. Fungi were isolated from Hg-polluted soils in tropical French Guiana. In addition, physiological parameters of fungal communities, such as the carbon source consumption, the acidification of the medium, and the production of low molecular mass organic acids (LMMOAs), were observed during the experiment.

2. Materials and Methods

2.1. Soil Sampling

For the isolation of Hg-resistant fungi from Hg-polluted soil, soil from French Guiana was used. The soil comes from the catchment basin of the Combat creek (1 km^2), situated approximately 10 km from the village of Cacao in French Guyana (52°230′ W, 4°350′ N). Soil profiles up to 1 m deep were sampled with a metal auger, and in each the first 50 cm

layer was immediately sealed in sterile hermetic polyethylene bags. These soil samples correspond to a ferralsol (WRB) and are contaminated by 0.5 ppm of Hg in the first 50 cm of depth [6]. These soils appeared to have been directly affected by illegal goldmining.

2.2. Isolation and Purification of Hg-Resistant Fungi

A total of 1 g of soil from a Hg-polluted site was mixed with 10 mL of sterile physiological water (NaCl 0.9 w/v) in a centrifuge tube (VWR) for 2 h. After centrifugation, 1 mL of the supernatant was sampled and diluted 10 times in sterile physiological water. Then, 100 µL of solution was spread on Melin Norkans agar plates spiked with 20 ppm of $HgCl_2$ and incubated at 24 °C for 2 days under aerobic conditions. The composition of 1 litre of Melin Norkrans medium is as follows: 50 mg of KH_2PO_4, 25 mg of $(NH_4)_2HPO_4$, 5 mg of $CaCl_2{\cdot}2H_2O$, 2.5 mg of NaCl, 15 mg of $MgSO_4{\cdot}7H_2O$, 1 mg of thiamine, 10 mg of $NH_4Fe^{III}(SO_4)_2{\cdot}12\ H_2O$, 10 g of glucose, 3 g of malt extract, and 15 g of agar. Several fungal colonies were isolated and purified by performing successive replicates on Petri dishes. Then, pure fungal colonies were stored at −80 °C until their utilization and identification in a specific medium (Melin Norkans medium diluted 10 times supplemented with 20% (w/v^{-1}) glycerol).

2.3. Identification of Hg-Resistant Fungi

Fungi identification was performed using 18S rRNA gene amplification and sequencing. The Fast DNA spin kit (MP Bio®, Illkirch, France) was used to extract DNA from each strain according to the manufacturer's instructions. Agarose gel electrophoresis (1%) was used to concentrate and purify the extracted DNA (2 µL), and the DNA amount was measured by microspectrophotometry (absorbance at 260 nm on a NanoDrop 1000, Thermo Fisher Scientific, Villebon Courtaboeux, France).

PCR was performed on 1 µL DNA extract and universal primers. The total reaction volume contained 1× Taq PCR Master Mix (Qiagen®, Courtaboeuf, France) and 0.1 µM of each primer (ITS4 5′-TCCTCCGCTTATTGATATGC-3′ and ITS5 5′-GGAAGTAAAAGTCGTAACAAGG-3′) at a total quantity of 15 µL. Before the sequencing was performed by MWG Biotech Company (Courtaboeuf, France), the PCR products were purified and concentrated using mini-columns (High PureTM PCR product Purification Kit, Roche diagnostic, Meylan, France). The Blast program was used to compare the sequences with those of the GenBank databases ("https://blast.ncbi.nlm.nih.gov/Blast.cgi" (accessed on 21 February 2024)).

2.4. Hg-Resistant Capacity and Bioaccumulation of Fungi

These two fungal strains were grown in Petri dishes on half-strength MMN medium for 2 weeks to obtain pure, fresh cultures of the fungi. Circular agar plugs (0.5 cm diameter) were cut out of the margins of the fresh cultures of the fungal colonies, and the fungal material was carefully separated from the agar. These circles of fungi were used as inoculum during all experiments in flasks.

The two fungal strains isolated were used to assess their resistant capacity. Experiments were performed using VWR® tissue flasks (75 cm^2, treated, vented cap, sterile). Each flask contained 200 mL of Melin Norkrans medium spiked with a solution of $HgCl_2$ to reach final Hg concentrations of 0, 1, 2, 3, 4, 10, 15 and 20 ppm. The flasks were agitated continuously on a VWR Advanced digital shaker at 100 rpm and 24 °C for 7 days in triplicate experiments. The EC_{50} value determination was performed with an online tool [39] ("https://www.aatbio.com/tools/ec50-calculator" (accessed on 17 April 2024)) that applied a nonlinear regression model with a three-parameter logistic curve. At the end of the experiment, the fungal biomass and the C-consumption by the fungi were also measured.

2.5. Biodissolution of Cinnabar Ore

The experiments were performed using VWR® tissue flasks (75 cm^2, treated, vented cap, sterile, Rosny-sous-bois, France) with the two Hg-resistant fungal strains (*Trichoderma koningiopsis* and *Talaromyces verruculosus*). Each flask contained 200 mg of cinnabar ore and 200 mL of sterilized MMN modified medium (without Fe, S, and Mg) to encourage the fungi to "seek" these elements necessary for their development. The composition of the modified Melin Norkrans medium (MMN) in one litre is 50 mg of KH_2PO_4, 25 mg of $(NH_4)_2HPO_4$, 5 mg of $CaCl_2 \cdot 2H_2O$, 2.5 mg of NaCl, 1 mg of thiamine, 10 g of glucose, and 3 g of malt extract. The pH of the medium was 6.5.

Before each experiment, fungi were transferred to Melin Norkans agar. After 2 weeks of growth, fungal plugs 8 mm in diameter were cut from the edges of the colony and used to inoculate each flask.

Flasks were agitated continuously on a VWR Advanced digital shaker at 100 rpm and 24 °C for 3 weeks in triplicate experiments. Solution samples were collected at 7, 14, and 21 days. The supernatants were then centrifuged, filtered at 0.2 µm (filter PTFE, VWR), and stored at 4 °C until analysis. Control experiments were performed under the same conditions (100 mL of modified MMN medium with the fungal strains) without cinnabar ore or Hg in the solution.

2.6. Abiotic Dissolution of Cinnabar Ore

A cinnabar ore pH-dissolution experiment was performed using VWR® tissue flasks (75 cm^2, treated, vented cap, sterile, Rosny-sous-bois, France) at pH 2, 3, 4, 5, and 6.5. Each flask contained 100 mL of sterilized MMN medium and 200 mg of cinnabar ore. The pH was adjusted by adding nitric acid. Flasks were agitated continuously on a VWR advanced digital shaker at 100 rpm at 24 °C for 7 days in triplicate experiments. Solution samples were collected at the end of the experience. The supernatants were then centrifuged, filtered at 0.2 µm (filter PTFE, VWR), and stored at 4 °C before analysis.

2.7. Cinnabar Ore Collection and Their Chemical Characteristics

Cinnabar ore was collected from the Almaden district in Spain (coordinates: 38.7206764766987,−5.49752113741748). The ore crystal was crushed and sieved (5 to 100 µm) then rinsed and sonicated with distilled deionized water to remove fine particles. The sample was then X-rayed to determine its mineralogical composition. This analysis was performed by the Alysés platform at IRD France-Nord (Bondy, France) using an X-ray diffractometer (DRX, X'PERT POWDER, Panalytical, Malvern, UK). Its chemical compositions, including cinnabar, quartz, pyrite, and Fe/Al oxy-hydroxides, such as goethite, are presented a study of the laboratory [40].

The cinnabar ore was also acid digested to quantify the proportion of Hg and Fe in the sample. This method consisted of dissolving 50 mg of cinnabar powder in a mixture of concentrated acids (3 mL HNO_3 and 3 mL HCl) for 48 hr at 80 °C. The mixture was then evaporated to dryness. All obtained residues were dissolved in 10 mL 5% HNO_3 before analysis by inductively coupled plasma optical emission spectrometry (SPECTROBLUE ICP-OES, SPECTRO Analytical, Kleve, Germany).

2.8. Chemical Composition Analyses

2.8.1. pH

pH of the medium was measured at each sampling event using a pH meter (apparatus 744 pH meter metrohm®, Villebon Courtaboeuf, France). In addition, the leached Fe in the solution was measured using ICP-OES (SPECTROBLUE ICP-OES, SPECTRO Analytical, Kleve, Germany). The detection limit for Fe was 0.005 ppm.

2.8.2. Hg Quantification

The Hg content in the solution was measured at the beginning and end of the experiment using an advanced mercury analyser (AMA-254®, Morlaàs, France) without sample pretreatment or pre-concentration. Each sample was burned in an oxygen-rich atmosphere (99.5%), and the evolved gasses were then transported using an oxygen carrier gas through specific catalytic compounds (to remove interfering impurities, e.g., ash, moisture, halogens, and minerals) to an Au-plated ceramic amalgamator, which collects the mercury in the vapour. The amalgamator was then heated to ~700 °C to release mercury to the detection system, which contained a Hg-specific lamp, emitting light at a wavelength of 253.7 nm. A silicon UV diode detector was used for mercury quantification. The working range was between 0.05 ng and 500 ng.

To assess Hg adsorbed in fungi cells, fungi biomass was put in contact with a solution of 10 mL of $CaCl_2$ (0.1 M) for 1 h. The biomass of each replicate was combined to obtain enough material. After centrifugation and filtration at 0.2 µm (filter PTFE, VWR), the Hg in the supernatant was analysed by AMA-254®. As a consequence of biomass combination, there were no replicates for this analysis.

2.8.3. Analyses of C-Consumption and LMMOA Production by Fungi

For C-consumption, sugar was used as a standard, and consumption was quantified using spectrophotometry [41]. In detail, 0.2 mL of the sample was mixed with 0.2 mL of 5% phenol, followed by the addition of 1 mL of concentrated sulfuric acid (98%). After stirring and 30 min of cooling, the OD was measured at 490 nm using a spectrophotometer (Genesys 10 UV scanning device®, Thermo Fisher Scientific, Villebon Courtaboeux, France). A calibration curve was made with glucose, which was implemented to calculate the amount of carbon in the solution from the degradation of sugars initially present in the mineral media (glucose and malt extract).

The LMMOA amount was measured using high-pressure liquid chromatography (HPLC) and the method was lightly modified from Van Hees et al., 1999 [42]. Samples were separated on a C18 stationary phase (AQUASIL C18, 5 µm, 250 × 4.6 mm) using a mobile phase of 1% ACN/99% 0.05 M KH_2PO_4 (pH 2.8) at a flow rate of 1.25 mL.min^{-1}. The column was operated at 20 °C to separate oxalic, malic, maleic, malonic, succinic, fumaric, and citric acids. The acids were detected at 210 nm by a UV detector.

3. Results and Discussion

3.1. Ability of Fungi to Grow in an Environment Polluted with Hg

The two fungal strains were identified with more than 99% similarity: *Trichoderma koningiopsis* (Accession number from ncbi: gb|JQ040370.1|; 554 bp) and *Talaromyces verruculosus* (Accession number from ncbi: gb|HQ607791.1|; 537 bp). These two fungi are known to be present in and highly tolerant of soil contaminated by heavy metals [43,44].

The fungal biomasses of *T. koningiopsis* and *T. verruculosus* and the consumed C are presented in Figure 1. These two fungal strains seem to have similar behaviour. For both strains, the fungal biomass decreased with increasing Hg content in the media. Without Hg, the biomasses of *T. koningiopsis* and *T. verruculosus* were approximately 70 and 63 mg dry weight (DW), respectively (Figure 1A). The fungal biomass greatly decreased, with an average of 44 and 24 mg DW for *T. koningiopsis* and *T. verruculosus*, respectively, in media containing 1 to 4 ppm Hg. The effect of Hg on fungal growth was observed at low Hg concentrations (1 ppm). At the end of the experiment, the fungal biomass in the media containing 10 and 20 ppm Hg was very low (5 mg DW). Our results were confirmed by the results of some authors [45], in which they showed that fungal colony development was disturbed at all Hg levels (from 0 to 20 ppm). In the media containing 10 to 20 ppm of Hg, these fungal strains had a high level of C-consumption, with an average of 82%

(Figure 1B), though low fungal growth was observed. In the media containing 0 to 4 ppm of Hg, 70% C-consumed was found. It seems that in the high-concentration Hg treatments, the fungi consumed more C than those in the low- or no-Hg treatments to provide the energy required to support their metabolism and biomass production under stressful conditions [46].

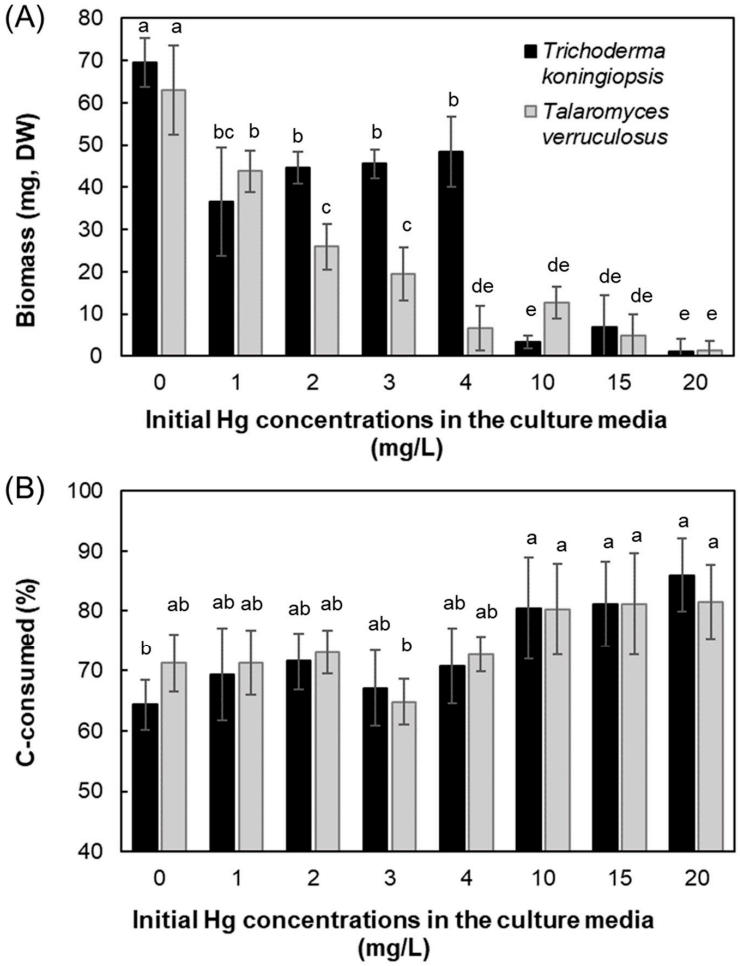

Figure 1. Biomass (mg, DW) (**A**) and C-consumed (%) (**B**) in the culture media at different Hg concentrations (0 to 20 mg/L) of the two fungal strains at the end of the experiments (7 days) (mean values ± SE, $n = 3$). Different letters (a, b, c, d and e) are significantly different ($p < 0.05$) according to Tukey's range test.

The EC_{50} values were determined as 6 mg.L^{-1} and 1.5 mg.L^{-1} for *T. koningiopsis* and *T. verruculosus*, respectively. These values are the same order of magnitude as those of the resistant bacterial strains in another study [47]. Moreover, Crane et al. (2010) [37] indicated that the biomass of ectomycorrhizal fungal strains, such as *A. muscaria*, *L. lacatta* and *P. tinctorius*, decreased by up to 50% in the presence of Hg at 1 mg.L^{-1} and by up to 75% in the presence of 5 mg.L^{-1} Hg in the media. This is in accordance with our results. Although the mechanisms of fungal resistance to Hg are not well known, some studies have suggested that the mechanisms involved are volatilization [29], absorption [48], and accumulation in fungal cells mediated by P-type ATPases [49].

The remaining Hg in the media of the *T. koningiopsis* and *T. verruculosus* cultures at the end of the experiment are presented in Figure 2A. Regardless of the fungal strain and the Hg concentration in the media, less than 0.07% of Hg was detected at the end of the experiment. This means that both fungal strains removed more than 99% of Hg in the media. We can highlight that these two fungal strains have a high potential for

Hg absorption or removal from the media. The Hg absorption of *T. verruculosus* was three-times higher than that of *T. koningiopsis* (Figure 2B). These results are consistent with the literature data [36,48,50]. However, there was no significant correlation between the Hg concentration in the media and the Hg absorption. Some studies have also shown that in field experiments, the accumulated Hg in mushroom tissues was not correlated with the Hg content in soil [9], and Hg absorption was very species specific. The Hg removed was due to absorption, accumulation, and volatilization by fungi (reduction of Hg^{2+} to metallic Hg^0 vapour) [36,51,52]. Nevertheless, in our experiments, Hg accumulation in fungi was measured by AMA-254®, Morlaase, France, but it was not possible to quantitatively measure Hg in the fungi because the detector was saturated every time, regardless of the experiment or the fungal strain. This saturation indicates that a portion of Hg accumulates in the fungal cells, and another portion of Hg can be adsorbed onto fungal cells. There was no correlation between fungal biomass and Hg adsorbed on cells. The capacity of Hg absorption by these two fungal strains in the present study was lower than those of four kinds of immobilized fungi residues in a previous study [53]. This can be explained by the different functional groups in different fungal strains promoting metal adsorption that are found in cell walls, such as hydroxide, amide, phosphate, and carboxylic groups [35,54]. Moreover, a study [54] highlighted that the optimal pH for Hg adsorption of Aspergillus versicolor was between 5 and 6. In the present study, the pH was approximately 3 during the experiment with these two fungal strains. This highlights the fact that Hg absorption depends not only on fungal strains but also on environmental parameters such as pH. In the future, the Hg volatilization capacity of these two fungal strains (*T. koningiopsis* and *T. verruculosus*) will be analysed to establish a complete mass balance for Hg.

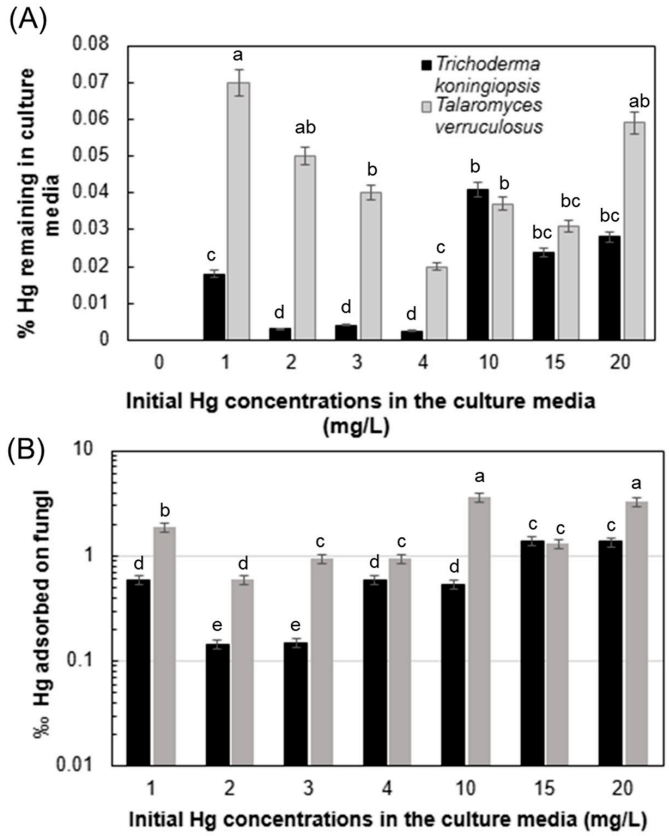

Figure 2. Hg remaining (%) in the culture media (**A**) and Hg adsorbed by the fungi (**B**) in each strain in the culture media at different Hg concentrations (0 to 20 mg/L) at the end of the experiments (7 days) (mean values ± SE, n = 3). Different letters (a, b, c, d, and e) are significantly different ($p < 0.05$) according to Tukey's range test.

3.2. Effect of Hg (Content and Availability) on Fungal Activities

A total of two-hundred milligrams of cinnabar ore were added during the biodissolution experiments; thus, in each experiment, there was potentially 110 mg of Hg in 200 mL of culture medium, reaching a final Hg concentration of 22 mg.L^{-1}. Cinnabar ore is very stable, and Hg is trapped in cinnabar, thus being less bioavailable than free Hg. In parallel, the same experiment was performed with $HgCl_2$ (final Hg concentration of 20 mg.L^{-1}) as in this form, Hg is bioavailable. Control experiments were performed under the same conditions but without Hg.

The fungal biomass of the two strains seemed to have the same behaviour, and there was no significant difference between the fungal strains according to the results obtained from a one-way ANOVA and the Tukey test ($p < 0.05$). The fungal biomass decreased with increasing Hg concentration in the medium. Without Hg, the biomasses of *T. koningiopsis* and *T. verruculosus* were approximately 85 and 68 mg, respectively (Figure 3). In the medium containing 20 mg.L^{-1} available Hg (in the form of $HgCl_2$), the biomasses of *T. koningiopsis* and *T. verruculosus* were very low (only approximately 9.7 and 8.7 mg, respectively (Figure 3)). The highest biomasses of *T. koningiopsis* and *T. verruculosus* were 249 and 268 mg, respectively, and were observed in the medium containing 22 mg.L^{-1} of cinnabar ore (without available Hg) (Figure 3).

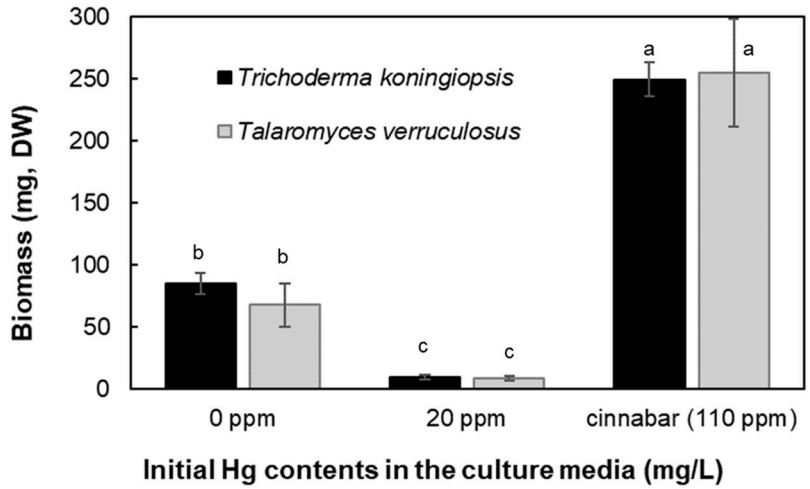

Figure 3. Biomass (mg, DW) in the culture media of each fungal strain containing bioavailable Hg at 0 and 20 mg/L^1 and cinnabar at the end of the experiments (21 days). Different letters (a, b, and c) are significantly different ($p < 0.05$) according to Tukey's range test.

The medium pH, which seemed to be modified by the LMMOAs produced by the fungi, was also measured during the experiments. The pH of the medium of *T. verruculosus* was lower (higher acidity) than that of *T. koningiopsis* (Figure 4A), regardless of the Hg concentration (except for at 0 ppm and in the cinnabar treatment) and experimental duration. For both fungal strains, the pH decreased during the experiment until the end of the experiment. At the end of the experiment, the medium became acidic (the pH decreases from 6.5 to approximately 4.0 and 3.0 for the *T. koningiopsis* and *T. verruculosus* cultures, respectively, at 0 ppm and in the cinnabar ore treatment). In the medium containing 20 ppm of available Hg, the medium also became acidic (the pH decreased from 6.5 to 4.7 and 4.6 for the *T. koningiopsis* and *T. verruculosus* cultures, respectively) but were not as acidic as those in the 0 ppm and cinnabar ore treatments.

The fungal activities were estimated by their capacity for C-consumption, pH, and the production of low molecular weight organic acids (Figures 4 and 5). The highest C-consumption by the two fungal strains was observed after 7 days of the experiment

(Figure 4A). The C-consumption was significantly different between the two fungal strains regardless of the conditions (0 ppm or 20 ppm of Hg, or cinnabar treatment). As was previously observed (see Section 3.1), regardless of the fungal strains, the highest C-consumption was observed in the medium containing 20 ppm available Hg. The production of LMMOAs, such as oxalic, citric, acetic, and formic acids, by these two fungal strains was measured in our experiments. Regardless of the conditions, *T. verruculosus* produced more LMMOAs than *T. koningiopsis*. In detail, acetic and formic acids were the most abundant in the medium containing 20 ppm of available Hg, whereas in the presence of cinnabar, oxalic and citric acids were produced at the greatest abundance. Several works [55,56] have shown that microbial communities tend to produce lactic, acetic, or formic acids when nutrient contents are high in their environment. Indeed, they require less energy to metabolize nutrients in high-nutrient environments than in a stressful environment to acquire a larger ATP generation. Nevertheless, the C source in our experiment seemed to be a factor limiting the production of acids. Moreover, the LMMOA content at 7 days was underestimated due to the degradation of these compounds by the fungal community. At 21 days of the experiment, the LMMOA content slightly increased but was always under the detection limit.

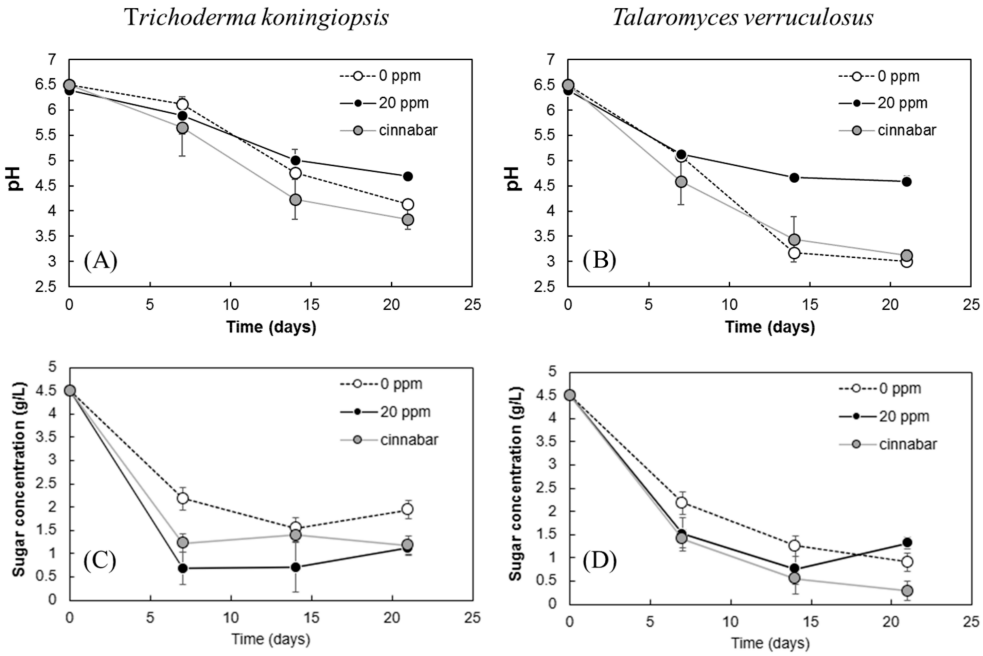

Figure 4. pH of the fungal strains *T. koningiopsis* (**A**) and *T. verruculosus* (**B**) as well as sugar concentration of the fungal strains *T. koningiopsis* (**C**) and *T. verruculosus* (**D**) during the experiments in culture media containing bioavailable Hg at 0 and 20 mg.L^{-1}, and cinnabar (mean values ± SE, n = 3).

From the data collected for biomass, LMMOAs, and C-content, we calculated the percentage of C-mineralized, which is presented in Figure 5. The C-mineralized corresponds to the ratio between the carbon-produced equivalent of biomass and LMMOA production and the C-used. These two fungal strains have the same tendencies regarding C-mineralization. The percentage of mineralized C was very low (0.14%) in the medium containing available Hg, whereas the percentage of C-mineralized was high, reaching 1.6% and 5% for the medium without Hg or that containing cinnabar, respectively. In other words, energy efficiency is not favourable under stressful conditions. The presence of Hg in the culture directly affected the catabolism and anabolism of the fungi, inducing low fungal growth, high C-consumption, and the production of LMMOAs. This could reduce the toxic impact of Hg on fungi. This result is supported by the significant correlation between

the contents of total Hg and bioavailability in the culture media and the fungal biomass and the production of LMMOAs (Table 1). A positive correlation was found between the production of acetic acid and formic acid and the Hg content. Fungi can decrease the accumulation of metals in the cytosol by producing chelating ligands and binding metals to cell-wall components [57,58].

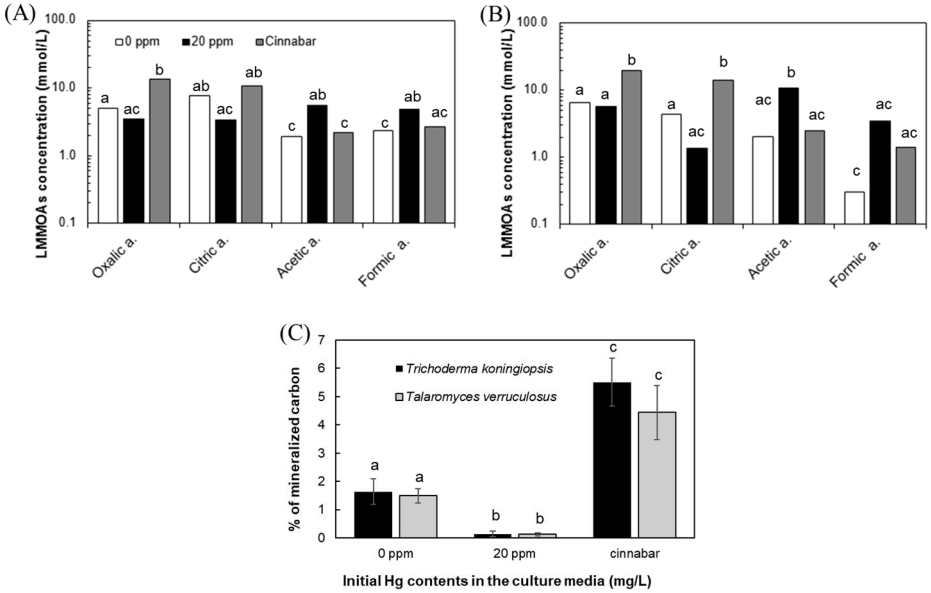

Figure 5. LMMOA concentrations (mmol/L) for *Trichoderma koningiopsis* (**A**) and *Talaromyces verruculosus* (**B**) and C-metabolized (%) in the culture media (**C**) with a bioavailable Hg content of 0 mg/L and 20 mg/Land cinnabar (no bioavailable Hg) after 7 days (mean values ± SE, n = 3). Different letters are significantly different ($p < 0.05$) according to Tukey's range test.

Table 1. Pearson correlation coefficients (n − 1) between total Hg contents, pH, biomass (mg DW), C-mineralized (‰), and LMMOA by-products of two fungal strains in the culture media (n = 18). Significant correlations are presented by numbers in bold according to the Pearson correlation coefficient.

Variables	Biomass	pH	Oxalic Acid	Citric Acid	Acetic Acid	Formic Acid	C-Mineralized	Total Hg Contents
Biomass	**1**							
pH	−0.100	**1**						
Oxalic acid	**0.932**	−0.382	**1**					
Citric acid	**0.951**	−0.129	**0.895**	**1**				
Acetic acid	**−0.582**	−0.406	−0.373	**−0.667**	**1**			
Formic acid	−0.411	−0.225	−0.413	−0.397	**0.573**	**1**		
C-mineralized	**0.831**	0.282	**0.676**	**0.851**	**−0.682**	**−0.516**	**1**	
Total Hg contents	**−0.699**	−0.423	**−0.538**	**−0.729**	**0.876**	**0.800**	**−0.871**	**1**

Significant correlations are presented by numbers in bold according to Pearson correlation coefficient.

3.3. Effect of Fungi on Hg and Fe Mobility During the Dissolution of Cinnabar Ore

As outlined in the results above, the fungal activity during the dissolution of cinnabar ore was linked to pH, C-consumption, and the low-molecular-weight organic acids produced. Additionally, the activity of the fungal strain *T. verruculosus* led to higher acidification of the medium and produced more LMMOAs than those produced by *T. koningiopsis*. Several studies [59–61] have noted a direct link between LMMOA production and mineral dissolution. Moreover, other studies [61,62] have highlighted an active strategy by the microorganisms to acquire nutrients. For example, oxalic and citric acids were preferentially produced to dissolve Fe from minerals. Based on this strategy, we supposed that the leaching of Hg was an indirect consequence. We suggest that LMMOAs produced by fungi acquire iron trapped in cinnabar ore by complexing with the iron. The ligand may not be selective and could also complex with Hg and, consequently, permit the leaching of mercury. This hypothesis is congruent with the litterature [63], which reports three pathways for the

chemical dissolution of cinnabar: (1) Hg^{2-} complexing ligands, (2) transformation of S^{2-} in cinnabar into soluble or volatile species and thus leaching of Hg^{2+}, and (3) displacement of Hg^{2-} in cinnabar by thiophile metals, such as Fe^{2+}.

In the present study, these fungal strains were permitted to leach Fe and Hg during cinnabar ore dissolution. The leaching of Fe and Hg have the same profiles (Figure 6). *T. verruculosus* was more efficient at dissolving cinnabar ore than *T. koningiopsis*. The ability of these fungi to leach Fe was effective in the first week and reached 0.8 and 0.9 ppm for *T. koningiopsis* and *T. verruculosus*, respectively. After that, the leaching of Fe was stable. For Hg, *T. koningiopsis* and *T. verruculosus* were able to leach 22 and 38 $\mu g.L^{-1}$ after 7 days of exposure. Then, a decrease in Hg leaching was observed for both fungal strains. These results are supported by the significant correlation between Fe and Hg leaching with the acidity of the medium and the parameters of fungal activity (Table 2). A positive significant correlation was found between the production of oxalic and citric acids, pH, and the leaching of Fe and Hg.

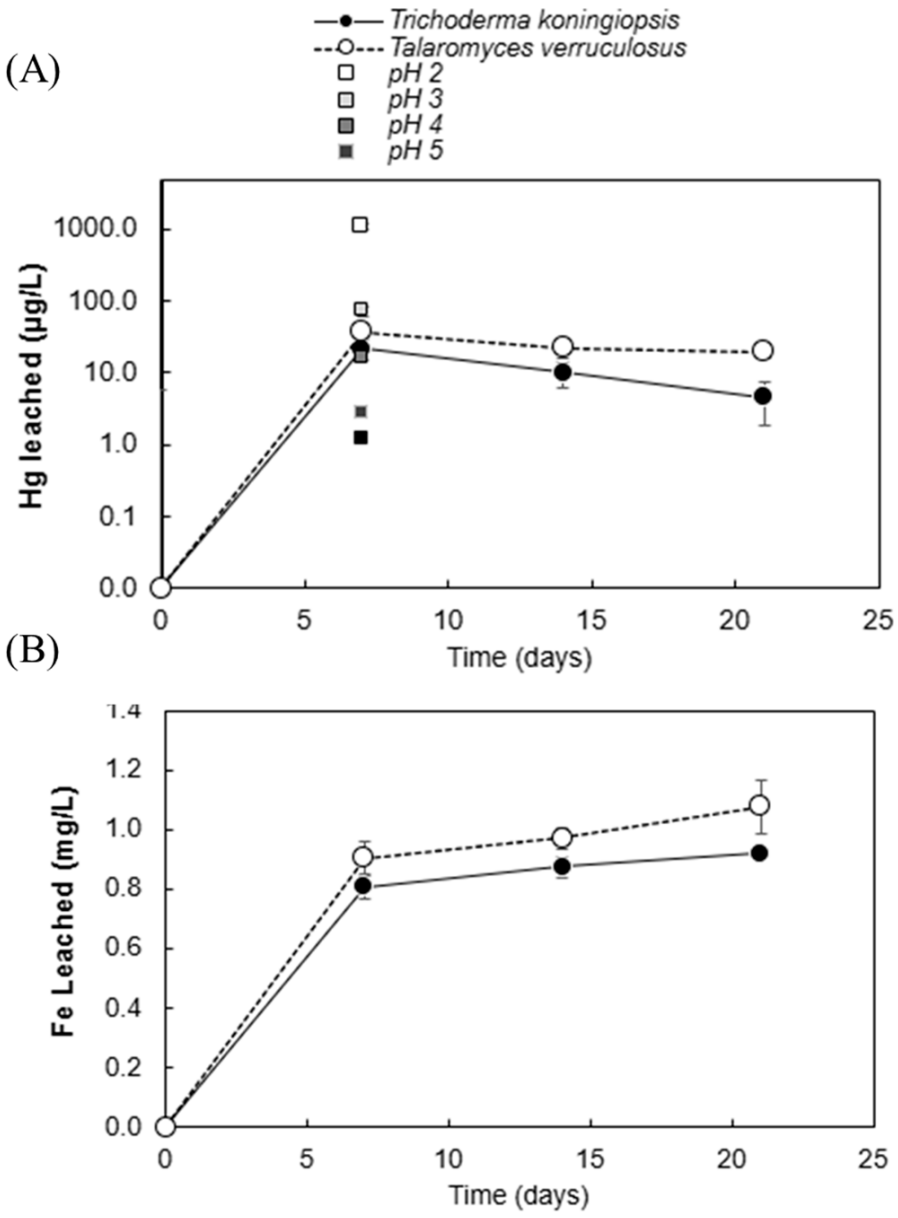

Figure 6. Leached Hg (**A**) in the culture with cinnabar and leached Fe (**B**) during the experiments for each fungal strain (mean values ± SE, n = 3). According to analysis of variance repeated measures and the Tukey test ($p = 0.05$) on three replicates, there is a significant difference between the two strains.

Table 2. Pearson correlation coefficients (n − 1) between Hg and leached Fe, pH, biomass, C-mineralized, and LMMOA by-products of two fungal strains during fungal biodissolution of cinnabar (n = 6). Significant correlations are presented by numbers in bold according to the Pearson correlation coefficient.

Variables	Biomass	pH	Oxalic Acid	Citric Acid	Acetic Acid	Formic Acid	C-Mineralized	Hg-Leached	Fe-Leached
Biomass	**1**								
pH	−0.604	**1**							
Oxalic acid	0.553	**−0.975**	**1**						
Citric acid	0.584	**−0.975**	**0.997**	**1**					
Acetic acid	0.674	**−0.930**	**0.941**	**0.963**	**1**				
Formic acid	−0.368	**0.905**	**−0.942**	**−0.915**	**−0.772**	**1**			
C-mineralized	0.243	0.291	−0.335	−0.268	0.003	0.633	**1**		
Hg- leached	0.585	−0.716	**0.820**	**0.808**	0.728	**−0.815**	−0.396	**1**	
Fe-leached	0.359	−0.593	**0.759**	0.617	0.429	**−0.811**	**−0.753**	**0.785**	**1**

Significant correlations are presented by numbers in bold according to the Pearson correlation coefficient.

In the medium at pH 2, the Hg released was 1000 times higher than that in the medium at pH 6.5. The rates of Hg release in our study were compared to those of the other works in the literature and are presented in Figure 7. The experiments performed in flasks with varying pH values with the addition of nitric acid allowed for the observation of a defining line representing the proton-promoted dissolution of cinnabar. These results were confirmed by some studies [64], in which the dissolution of cinnabar was performed with HCl at pH 2. The data collected are in line with our results, validating our model of the proton-promoted dissolution of cinnabar.

In the medium at pH 4.5–5.5, the rates of Hg release by these fungal strains were 10 times higher than those observed for nitric acid. Fungi are well known for their capacity to weather cinnabar and are more efficient than bacteria [40], with increasing efficiency in the presence of organic matter [65]. Nevertheless, regardless of cinnabar dissolution, the rates of Hg dissolution were underestimated due to bioaccumulation [7,37,48]. Indeed, we have already shown that the high capacity of Hg absorption of these two fungal strains led to a decrease in Hg in the medium. We supposed that the rates of Hg dissolution are also linked to the capacity of Hg absorption by microorganisms [53], the organic matter and iron oxy-hydroxide content [66,67], and the volatilization of Hg [36,51]. Fungi could be an important factor for the leaching of cinnabar ores to extract the metals of interest. They could also be used for metal depollution due to their capacity in metal transformation and bioaccumulation.

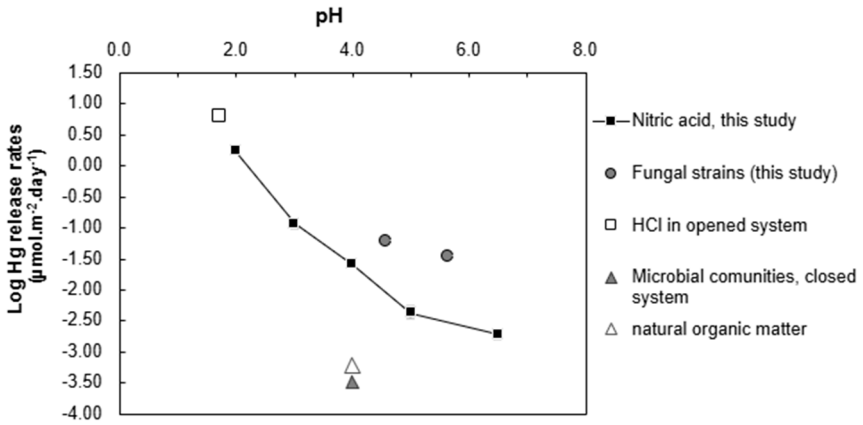

Figure 7. Log Hg release rates (µmol.m^{-2}.day^{-1}) versus pH for abiotic dissolution of cinnabar ore (HNO_3), for biotic experiments (fungal strains), and the literature data (HCl in opened system [64], Microbial communities in closed system [40] and natural organic matter [65]).

4. Conclusions

Two fungal strains are isolated from Hg-polluted soils in French Guiana and identified as *Trichoderma koningiopsis* and *Talaromyces verruculosus*. These fungal strains possess a

high capacity for Hg resistance and for removing over 99% of Hg content in the culture media by the end of the experiment. Fungal biomass decreases with increasing mercury concentration. The production of organic acids by fungi is observed for both fungal strains, leading to an acidic pH in the medium culture. Oxalic and citric acids are preferentially produced to dissolve Fe from minerals, which may impact Hg leaching. The results of this study provide evidence that the two fungi seem to have potential use for the bioremediation of Hg during the dissolution of cinnabar ores through biosorption mechanisms.

Author Contributions: Conceptualization, C.B. and M.D.J.; methodology, V.A. and S.A.-A.; software, C.B. validation, C.B. and V.A.; formal analysis, C.B.; investigation, C.B. and M.D.J.; resources, C.B. and N.B.; data curation, C.B.; writing—original draft preparation, C.B.; writing—review and editing, C.B. and M.D.J.; visualization, C.B.; supervision, N.B.; project administration, N.B.; funding acquisition, N.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ANR Interconnect, grant number ANR-11-CESA-0020.

Data Availability Statement: Data are available on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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