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Research Article

Fungi that Survived in a Contaminated Uranium Mine from Brazil

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Abstract

The Osamu Utsumi mine was the first uranium mine in Brazil and ceased activities in 1995. Since then, it has encountered problems in rehabilitating the mine site due to high levels of contamination. Thus, the present investigation aimed to isolate and identify fungi from the Osamu Utsumi mine that may be suitable candidates for the bioremediation process and thus get to know the local mycobiota. The pH of the water samples was 3.3. The mean water activity (a_w) of soil samples was 0.98. From this extreme environment, a total of 57 fungal species were isolated and identified, with genus *Penicillium* being the most abundant. The identification by classical techniques demonstrated that these fungi, despite being of the same species, in many cases, present different morphology. The accurate knowledge of the mycobiota capable of growing and survive on this type of environment, which fungi are dominant, provides essential data to help in futures studies. Effluents from the contaminated uranium mine can pose severe risks to the health of the population, as they can contaminate rivers and lakes close to the mine area, so it is necessary to properly treat the water from the acid mine drainage. In the future, the fungi isolated can potentially be used in the bioremediation process, providing an economical and eco-friendly alternative to conventional treatment.

Keywords: Fungi; Biodiversity; Uranium mine; Penicillium; Waste

Introduction

Considerable amounts of uranium can be mobilized and released into the environment as a consequence of the uranium mining and milling operations associated with the nuclear industry [1]. Pathways of exposure to uranium and associated contaminants may be direct or indirect. Direct exposures may occur by eating or drinking contaminated material, breathing dust or radon gas, skin contact, or from gamma-ray emissions. Indirect exposures occur when the uptake of uranium occurs by, for example, worms, insects, or plants, which then enter the food chain and may eventually enter the diets of humans [2,3]. Typically, ingestion and inhalation the primary routes of entry into the body [4].

The most significant exposure pathways for ecological resources occur *via* surface waters. Such water may contain high concentrations of radionuclides, particularly if waste treatment systems do not perform as designed. Thus, the environment can be contaminated. High levels of uranium in humans have been reported to affect the kidneys and bone marrow [5]. Studies suggest a strong correlation between environments with high uranium concentrations and a high prevalence of leukemia in local inhabitants, with uranium intake, mainly linked to the consumption of well water, animal meat, and the inhalation of dust [6].

In Brazil, Osamu Utsumi mine was the first uranium extraction and processing site operated by Indústrias Nucleares do Bra-

sil (INB). The mine is located in Caldas city, in the state of Minas Gerais, and began operation in 1982. In 1995, the mine entered in the decommissioning phase, but still requires large efforts for environmental reclamation [7,8].

During exploration for uranium and the initial mine stripping operations, barren waste rock was sent to sites around the open-pit mine (OPM). These tailings piles are referred by the term "Bota-Fora" (BF), being BF-4 and BF-8 (volumes of 12.4 and 14.8 million m^3, respectively) considered the most important [7]. These piles of contaminated tailings contain high concentrations of pyrite (FeS_2) which contributes to the formation of acid mine drainage (AMD).

AMD causes an increase in the concentration of heavy metals and radionuclides in rivers and lakes around the mine area [9]. To the control the flow of AMD, basins (BNF and BIA) have been constructed to capture the drainage water flowing from the BF-4 and BF-8 tailings piles. Contaminated water at the mine site is treated by the addition of calcium hydroxide to increase the pH and precipitate the heavy metals. The treatment generates a waste (alkaline mud) it is pumped back in the formed OPM created during the excavation of the mine.

Bioremediation has been proposed to improve upon or substitute for classical technologies for the remediation of contaminated areas [10]. The principle of this process is the removal of metals and radionuclides from contaminated environments using microorganisms to sequester contaminants or transform them into a less-toxic state.

Fungi have a high potential for bioremediation and the ability to accumulate and immobilize, at high concentrations, a variety of heavy metals such as Cu, Zn, Fe, U, Ni, Cd, Pb and Hg [11,12]. The potential of fungal biomass as adsorbents for the removal of heavy metals and radionuclides from polluted waters has been recognized [13-16]. Indigenous fungi under stress in extreme environments develop mechanisms of resistance and tolerance to xenobiotics agents, such mechanisms can be used in biotechnology research, as in the bioremediation of toxic metals.

In Brazil, problems related to the disposal and treatment of waste are very frequent, for example the recent environmental tragedies of Mariana and Brumadinho in Minas Gerais, where after the rupture of containment dams, tons of mining tailings have caused destruction and contamination of sites and immeasurable damage to life and the environment. The population living close to the uranium mine area can be contaminated by heavy metals in the soil and acidic water, despite the treatment and construction of tailings containment basins, significant amounts of heavy metals can contaminate effluents and rivers that are used for the supplying thousands of people.

In this study, we screened fungi in wastes samples in uranium mine from Brazil in order to understand which indigenous fungi grow in these contaminated environments.

The accurate knowledge of the mycobiota capable of growing and survive on this type of environment, which fungi are dominant, provides essential data to help in futures studies. These microorganisms can be used in uranium mycoremediation analyzes and other heavy metals, providing economical and eco-friendly alternative tools for the treatment of uranium-contaminated water. We also highlight the pioneering of this research in the mine area.

Material and Methods

Sampling collection

The sampling was carried out in Osamu Utsumi mine located in Caldas, state of Minas Gerais, Brazil. Samples of soil, water and sediment were collected from the main basins with acid water and waste piles. The collection points are shown in figure 1 and explained in the table 1. During sampling, the marking of collection points was performed by GPS (Global Positioning System).

Four soil samples (2 kg each), 3 water samples (3L each) and 2 sediment samples (2 kg each) were collected. A Van Veen collector was used to collect sediment from the bottom of the lake in the open-pit mine.

Figure 1: Osamu Utsumi mine, sampling locations are indicated as dark dots.

Sample collection locations are described below (Supplementary figures):

- "Bota-fora" 8 (BF-8) and "bota-fora" 4 (BF-4) tailings are mountains of waste rock/soil produced during the excavation of uranium ore.
- "BIA and BNF" are basins built in the mine area to limit the spreading of AMD. The BIA and BNF basins receive acidic water coming from tailings pile BF-8 and BF-4, respectively.
- "DUCA" is an alkaline mud waste formed during the treatment of water in the "treatment plant". The DUCA has been discarded in the open-pit mine.
- "OPM" (open-pit mine) is where the extraction of uranium ore took place. The OPM is currently flooded with acidic water to form a lake. The edge of the OPM is used for the disposal of the DUCA waste.

No. sample	Collection site	Sample type	GPS coordinate
1	BF-4	Soil	21°56'27.0S 46°29'21.8W
2	BF-8	Soil	21°57'14.4S 46°30'33.1W
3	BIA	Soil	27°57'28.2S 46°30'37.5W
4	OPM	Soil (edge)	21°56'48.8S 46°30'13.2W
5	BIA	Water	21°57'27.8S 46°30'37.3W
6	OPM	Water (lake)	21°56'46.4S 46°29'57.7W
7	BNF	Water	21°56'27.6S 46°29'23.9W
8	DUCA	Sediment	21°57'24.4S 46°30'29.9W
9	OPM	Sediment (bottom)	21°56'46.4S 46°29'57.7W

Table 1: List of the samples, collection sites, sample types and their GPS-coordinates.

Water activity (a_w) and pH

The a_w of soil samples was determined using an AQUALAB CX-2 water activity meter, Decagon Devices Inc (Pullman, WA, USA). The pH of the water samples was measured at the moment of sampling by digital pH meter (Kasvi).

Fungi isolation and identification

Sediment and water samples were placed directly on Petri plates (90 x 15 mm) containing Potato Dextrose Agar (PDA) (Oxoid, Basingstoke, UK) supplemented with chloramphenicol. For soil samples, 10g of soil was mixed with 90 mL of sterile distilled water (dilution 10^{-1}) and serial dilutions were prepared up to a dilution of 10^{-5}. From each dilution, an aliquot of 0.1 mL was inoculated onto plates containing PDA. Triplicate plates were incubated at 25°C for 7 days in the dark. The quantification of fungi was expressed in colony forming units per gram (CFU g^{-1}) for solid samples and CFU mL^{-1} for liquid samples [17,18].

The fungal isolates were identified at genus level by morphological features [19-22], were maintained in 15% glycerol stocks at −80°C in the Department of Microbiology at the University of Sao Paulo.

Molecular identification

DNA was extracted directly from fungal colonies grown on yeast extract sucrose agar (YES) [23,24] using the PrepMan Ultra® kit protocol (Applied Biosystems, Carlsbad, CA, USA). The DNA was quantified using the NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The internal transcribed spacer (ITS region) of the rRNA, widely used in molecular studies of fungi, has been selected as the formal barcode marker for fungi, the conserved ITS region is located between the 18S and 28S rRNA genes, includes two variable regions (ITS1 and ITS2) and the highly conserved 5.8S rRNA gene [25].

The universal primers indicated for fungal amplification are ITS1 (5′ TCC GTA GGT GAA CCT GCG 3′), which hybridizes at the end of 18S rDNA, and ITS4 (5′ TCC TCC GCT TAT TGA TAT 3′) which hybridizes at the beginning of 28S rDNA. The primers indicated for beta-tubulin gene are Bt2a (5′ GGT AAC CCA ATC GGT GCT GCT TTC 3′) and Bt2b (5′ ACC CTC AGT GTA GTGACC CTT GGC 3′). The fragments of ITS region of rDNA and beta-tubulin gene (*BenA*) were amplified with the primer pairs (Invitrogen, life technologies, CA, USA). Beta-tubulin protein-coding gene, involved in the generation of microfilaments, are more variable than ITS region, thus, is often used as a marker that allowing closely related Penicillium lineages to be discriminated [26,27].

The PCR mixture contained 12.5 µL 2 × PCR Master Mix (Promega, San Luis Obispo, CA, USA), 6.5 µL Milli-Q water, 2 µL DNA

(40 ng), and 2 µL (20 pmol) of each primer (Prodimol Biotecnologia, Minas Gerais, Brazil) in a final volume of 25 µL. The amplification program included an initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 57°C (ITS) and 55ºC (*BenA*) for 1 min, and extension at 72°C for 1 min. A final extension step at 72°C for 7 min was included at the end of the amplification.

The amplification products were purified with the QIAquick PCR Purification kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The PCR products were sequenced using the same primers as those employed for amplification with the Big Dye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). The reactions were run using a 3100 DNA sequencer (Applied Biosystems). Consensus sequences were obtained using the software Sequencher version 4.1.4 (Gene Codes Corporation, Ann Arbor, MI, EUA) and were used to perform BLASTn searches to confirm preliminary identifications at the NCBI (http://blast.ncbi.nlm.nih.gov/) and MycoBank (http://www.mycobank.org/) websites. The nucleotide sequences were deposited in the GenBank database [25,28,29].

Due to the limitations associated with the sequencing of the ITS region to identify the *Penicillium* genus, it is recommended that a second gene be used to confirm the identification of the species, with beta-tubulin indicated as being the best option for this [27].

Fungi isolated from Osamu Utsumi mine were compared with those found in GenBank. In the database, all the isolated fungi had a similarity ranging from 99 to 100% and an e-value ≤ 0 with the related fungi recorded in the bank.

Data Analysis and phylogenetic tree

All the nucleotide sequences were aligned with those corresponding to closest matches from GenBank using MUSCLE software included in the MEGA 7.0.26 package. Aligned data sets were analyzed using the maximum likelihood method with the Kimura 2-parameter model, the bootstrap analysis used 1000 replicates [26].

Results and Discussion

pH, water activity

The pH values of the water samples from Osamu Utsumi mine was ultra-acidic (pH 3.3 ± 0.1). AMD is responsible by the acidity of water samples, because the natural oxidation of metal sulfides (exposed in the pit, waste rock and tailings dam). As a result, sulfuric acid is formed, pH reduction and dissolution of heavy metals associated to rocky matrices with severe environmental impacts [30].

The National Environmental Quality Standards (NEQs) indicated the permissible limit for the pH of wastewater range from 6.5 - 8.5. Therefore, our results showed that pH values of all the wastewater samples were found above the permissible limit by NEQs-WHO. Water pH analysis is crucial to indicate the degree of water corrosion, according to the WHO the lower the pH, the higher the level of corrosion. Water pH lower than 4 may cause eye and skin irritation in humans [31].

Two of the most important environmental parameters that determine the ability of molds to grow in substrates are a_w and temperature. Usually, many filamentous fungi species shows excellent growth capacities at temperatures close to 25ºC [32,33]. In the mine area on the day of sample collection the temperature was 25ºC, this ambient temperature probably increases the survival capacity of isolated fungi in this extreme environment.

Water activity is a factor that can predict which fungal species will predominate in the environment. The a_w mean of soil samples was 0.98, a value which favors the growth of several species of filamentous fungi [33,34].

In previous studies carried out by our research group, uranium concentrations measurement of soil, water and sediment samples, were soil 50 to 245 mg kg^{-1}; water 1.05 to 4.46 mg L^{-1}; sediment 268 to 577 mg kg^{-1} [35]. The water samples showed uranium levels up to 200 times above the maximum permissible value (0.02 mg L^{-1}) by Brazilian environmental legislation.

Comparing the maximum levels established by Brazilian legislation with those of other countries, the values are very similar, ranging from 0.01 to 0.03 mg L^{-1} in Canada, Germany and the United States [36-38]. Uranium can be toxic to organisms even at low concentrations. According to the WHO Drinking Water Quality Guideline, the permitted limit for uranium in drinking water is 0.015 mg L^{-1}.

In the most of countries there is no resolution that establishes maximum limits for uranium concentrations in soil and sediment. In the literature, the only published acceptable limits of uranium in soil have been established by the Canadian Council of Ministers of the Environment (CCME), which establishes the maximum limit of

uranium in agricultural, residential and industrial areas of 23, 23 and 300 mg kg^{-1}, respectively.

Researches conducted in Germany and the United States indicates a strong relationship between the consumption of water with high uranium concentrations and the increase in the incidence of various types of cancer such as lung, colorectal, kidney, prostate, thyroid, and leukemia [36,39,40].

Fungal diversity

A total of 57 fungal isolates, from 14 genera, were identified at the species level by classical and molecular identification methods.

The Colony-Forming Units per gram (CFU g^{-1}) from soil samples ranged from 15 x 10^2 to 12 x 10^6 CFU g^{-1}; values in water were from 0 to 4 x 10^3 CFU mL^{-1}; and in the sediment samples ranged from 1 x 10^2 to 8 x 10^2 CFU g^{-1} (Table 2).

No. sample	Collection site/ Sample type	CFU g^{-1} or CFU mL^{-1} average ± (SD)	Amount of fungus	Species
1	BF-4 - soil	11 x 10^3 ± (3.5)	1	*Aspergillus* sect. *versicolores*
			2	*Talaromyces amestolkiae*
			1	*Trichoderma koningiopsis*
			1	*Umbelopsis ramanniana*
			2	*Penicillium pulvillorum*
			1	*Penicillium amphipolaria*
			2	*Penicillium ludwigii*
			1	*Penicillium citrinum*
2	BF-8- soil	15 x 10^2 ± (1.0)	1	*Talaromyces loliensis*
			3	*Talaromyces amestolkiae*
			1	*Tolypocladium album*
			1	*Purpureocillium lilacinum*
			1	*Metarhizium robertsii*
			1	*Pochonia chlamydosporia*
			1	*Verticillium leptobactrum*
			1	*Gongronella butleri*
			2	*Penicillium pulvillorum*
			1	*Penicillium brasilianum*
			1	*Penicillium amphipolaria*
			1	*Penicillium citrinum*
3	BIA - soil	5 x 10^5 ± (2.7)	1	*Aspergillus* sect. *versicolores*
			1	*Trichoderma koningiopsis*
			2	*Purpureocillium lilacinum*
			1	*Penicillium piscarium*
4	OPM - soil	12 x 10^6 ± (1.8)	1	*Talaromyces amestolkiae*
			1	*Trichoderma asperellum*
			1	*Bionectria ochroleuca*
			1	*Phoma* cf. *nebulosa*
			1	*Mucor cicirnelloides*
			1	*Mucor fragilis*
			2	*Penicillium piscarium*
			1	*Penicillium ochrochloron*
			1	*Penicillium brasilianum*
			1	*Penicillium janthinellum*
			1	*Penicillium citrinum*
5	BIA -water	-	-	Fungi not isolated

6	OPM - water	$4 \times 10^3 \pm (4.0)$	3 1	*Penicillium piscarium* *Penicillium citrinum*
7	BNF - water	$1 \times 10^3 \pm (4.7)$	1	*Penicillium citrinum*
8	DUCA - sediment	$1 \times 10^2 \pm (2.1)$	1	*Penicillium piscarium*
9	OPM - sediment	$8 \times 10^2 \pm (2.6)$	6 1 1	*Penicillium piscarium* *Penicillium janthinelum* *Penicillium citrinum*

Table 2: Identification of fungi isolated from soil, water and sediment samples, CFU g^{-1}, CFU mL^{-1} and average + standard deviation.

The low amount of fungi isolated from sediment and water samples can be attributed to the acid water. The high concentrations of uranium in the sediment samples [35] and the fact that the samples were collected from the bottom of an acid lake (in the OPM) may also have interfered with the isolation of fungal species from these samples. In comparison, fungi were more abundant in the soil samples. Radionuclide contamination may change fungal communities in the environment, including an increased proportion of melanized fungi and a reduced diversity of species [41].

The ITS and beta-tubulin phylogenetic tree of the fungi species isolated from Osamu Utsumi mine can be check out in figure 2 and 3, respectively. The sequences of amplicons from ITS region (rRNA) and beta-tubulin (rDNA) were highly similar to the sequences of the respective species of closely related fungi documented in GenBank®. Thus, in our findings we have determined homology from the degree of similarity between strains found within the database and our isolates.

Figure 2: Neighbor-joining phylogenetic tree (ITS region) of fungi species isolated from Osamu Utsumi uranium mine (isolated species identified by USPMCT+number) reference strains of corresponding fungi were included in the tree. *Trichoma paradoxa* CBS 103.73 was used as an outgroup.

Nucleotide sequences of the ITS and beta-tubulin region of 18s rRNA genes of isolated fungal were deposited in GenBank database and numbers are shown in table 3.

A total of 25 different species were isolated, with the greatest fungal diversity observed in the soil samples, where 20 different species were isolated. Low diversity was found in the water and sediment samples, being 2 and 3 different species, respectively, all belonging to the genus *Penicillium*.

Penicillium genus was the most isolated in the soil samples from the Osamu Utsumi uranium mine, were isolated 7 different species (Table 2), being *P. piscarium* the most frequent. Classical identification analyzes showed that, despite being of the same species, the fungi often demonstrated different morphology despite growing under the same conditions of temperature, humidity, and nutrients (Figure 4).

Previous studies have isolated *Penicillium* species in soil samples contaminated with high concentrations of heavy metals, possibly indicating that these fungi can better adapt to the selective pressure in these contaminated environments [42-45]. Authors have investigated *Penicillium* spp. isolated from uranium mines for bioremediation processes, because this high resistance and bioaccumulation of uranium and other heavy metals [46,47].

Figure 3: Phylogenetic tree (*BenA* gene region) of *Penicillium* species isolated from Osamu Utsumi uranium mine (isolated species identified by USPMCT+number). reference strains of corresponding fungi were included in the tree. *Penicillium tularense* CBS 43169 was used as an outgroup.

Figure 4: Morphological identification of isolated fungi in the Osamu Utsumi mine.

Isolates ID	Species	GenBank access number. ITS/*BenA*
USPMCT76	*Aspergillus* sect. *versicolores*	MH137656
USPMCT130		MH137674
USPMCT68	*Talaromyces loliensis*	MH137651
USPMCT42	*Talaromyces amestolkiae*	MH137642
USPMCT63		MH137650
USPMCT73		MH137654
USPMCT109		MH137665
USPMCT148		MH137680
USPMCT165		MH137688
USPMCT111	*Tolypocladium album*	MH137667
USPMCT72	*Trichoderma koningiopsis*	MH137653
USPMCT127		MH137672
USPMCT40	*Trichoderma asperellum*	MH137640
USPMCT53	*Purpureocillium lilacinum*	MH137646
USPMCT129		MH137673
USPMCT131		MH137675
USPMCT69	*Metarhizium robertsii*	MH137652
USPMCT135	*Bionectria ochroleuca*	MH137677
USPMCT104	*Pochonia chlamydosporia*	MH137664
USPMCT156	*Verticillium leptobactrum*	MH137684
USPMCT136	*Phoma* cf. *nebulosa*	MH137678
USPMCT74	*Umbelopsis ramanniana*	MH137655
USPMCT54	*Gongronella butleri*	MH137647
USPMCT47	*Mucor cicirnelloides*	MH137644
USPMCT43	*Mucor fragilis*	MH137642
USPMCT9	*Penicillium piscarium*	MH724302
USPMCT13		
USPMCT16		
USPMCT19		
USPMCT21		
USPMCT26		
USPMCT79		
USPMCT88		
USPMCT99		
USPMCT114		
USPMCT132		
USPMCT153		
USPMCT169		
USPMCT62	*Penicillium pulvillorum*	MH724303
USPMCT144		
USPMCT159		
USPMCT161		
USPMCT38	*Penicillium ochrochloron*	MH724304
USPMCT44	*Penicillium brasilianum*	MH724305
USPMCT56		
USPMCT81	*Penicillium amphipolaria*	MH724306
USPMCT102		
USPMCT94	*Penicillium ludwigii*	MH724307
USPMCT124		
USPMCT23	*Penicillium janthinellum*	MH724308
USPMCT152		
USPMCT110	*Penicillium citrinum*	MH724309
USPMCT115		
USPMCT122		
USPMCT151		
USPMCT158		
USPMCT167		

Table 3: Fungi isolated from Osamu Utsumi uranium mine with Genbank acess number.

Trichoderma was also isolated in this study. Durand., *et al*. 2017 reported *Trichoderma* as a dominant fungus in a Hg contaminated soil. *Penicillium* and *Trichoderma* are highly competitive genus due to their ability to outcompete other microorganisms, produce abundant conidia and survive in most environmental conditions [49].

Other fungal genera such as *Aspergillus*, *Talaromyces*, *Tolypocladium*, isolated from the mine environments, are also cited in the literature as resistant to heavy metals and other extreme conditions, this give them a high capacity for bioremediation [50-53].

In our experiments, no fungi were isolated from water samples in the BIA basin, and the amount of fungi isolated was lower in water and sediment samples when compared to soil samples. The low isolation of fungi in water can be attributed to the pH of the samples, also because the water mycobiota is transient.

According to Plumridge [54], acid pH plays an important role in inhibiting some fungal species. The optimum pH for the growth of filamentous fungi is close to 5, but there are fungal species that may adapt to a pH ranged from 1.5 - 11.

In addition to the low pH and high uranium concentrations in sediment samples, the fact that these samples were collected from the bottom of the acid lake, it causes a reduction in oxygen, this may have caused the low isolation of fungal species on these samples [55].

The most isolated fungi in our study have an adaptive advantage that guarantees their ecological advantage in stressful environments and locations with a lack of nutrients. The hypothesis is that in contaminated environments there are "signs" that can trigger the production of conidia, dispersion and survival of filamentous fungi in the environment, these signs can be light, mycelial lesions, low pH, minerals and heavy metals [56-58].

As noted, the local of the mine is very selective environments, characterized by extreme conditions to life. So, the fungal isolated from this mine are strongly adapted to acidic medium and high uranium concentrations.

The fungi isolated from the tailings piles in Osamu Utsumi mine have a high potential to be used in bioremediation processes, as previously demonstrated by our research group, where eleven of the fungal isolates were analyzed as promising candidates for mycoremediation being able to remove more than 60% of uranium from solutions: *Penicillium piscarium*, *Gongronella butleri*, *Phoma nebulosa* and *Talaromyces amestolkiae* species [35]. Mycoremediation using indigenous fungi is considered an economical and eco-friendly alternative to conventional treatments for the decontamination of sites with uranium and other heavy metals.

Conclusion

The water from Osamu Utsumi mine is highly acid. Fungal biodiversity analysis revealed that greater quantity and diversity of fungi were isolated from soil samples. A total of 57 fungal isolates, from 14 genera, were identified at the species in soil, water, and sediment samples. Although *Talaromyces*, *Trichoderma*, *Aspergillus*, and other species were isolated, most fungi belonged to the genus *Penicillium*, being the most prevalent the *Penicillium piscarium*. Inhospitable environmental conditions can cause stress on microorganisms and select the most suitable to survive in the environment. Thus, it is important to know which fungi are capable of growing and survive on this type of environment, these can be important as an alternative to conventional processes in the treatment of uranium contaminated environments. The accurate knowledge of the mycobiota capable of growing and survive on this type of environment and which fungi are dominant provide important data to help in advanced study. In the future, the fungi isolated can potentially be used in the bioremediation process, providing an economical and eco-friendly alternative to conventional treatment.

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Authors Contributions

E.C. and B.C. conceived the experiments. E.C., T.A.R. and R.C.O carried out experiments. E.C. and B.C. did modeling. E.C., T.A.R., R.C.O and B.C. wrote the manuscript. All authors discussed the data and the results, and commented the manuscript. B.C. supervised the project.

Competing Interests

The authors declare no competing financial interests and non-financial interests.

Supplementary figures

Figure 1: Panoramic view of the Osamu Utsumi open-pit mine (Lake with acid water pH 3.3).

Figure 2: Open-pit mine with the local where DUCA (dark mud) is dumped.

Figure 3: Effluent from the pile tailings bota-fora 4.
Acid water produced by the acid mine drainage is drained to the BNF basin.

Figure 4: Basin BNF collect acid water from Bota-for a 4.

Figure 5: Bota-fora 8 pile tailings.
Acid water from bota-fora 8 is drained to BIA basin.

Figure 6: Osamu Utsumi Uranium Mine Water Treatment Plant.
After the water treatment, the formed alkaline mud (DUCA) is discarded at the edge of OPM.

Figure 7: BIA basin located at Osamu Utsumi uranium mine.

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