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Radiotrophic fungi and their use as bioremediation agents of areas affected by radiation and as protective agents

Fungos radiotróficos e seu uso como agentes biorremediadores de áreas afetadas por radiação e como agentes protetores

Hongos radiotróficos y su uso como agente biorremediador de zonas afectadas por radiación y como agentes protectores

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Abstract

Nuclear waste, from nuclear fuel and nuclear accidents, represents a great risk to the environment and human beings, causing several problems such as malformation, and cancer, it can turn areas uninhabitable, causing changes in the fauna and flora of entire areas. In the search for a more effective way to deal with such contaminants, it is proposed the use of radiotrophic fungi, such as those found inhabiting the reactor of Chernobyl in Ukraine, the poles, and the international space station, due to their high resistance to these contaminants, the power of absorption of ionizing radiation and the deposition of radioisotopes in their cell walls, making possible the removal of them from the environment in which they are found, as well as the consumption of organic matter, such as the graphite present in the reactor number 4 of Chernobyl. Another property to be explored is the protective use of these organisms to reduce the incidence of ionizing radiation in areas of interest and protect human beings. Therefore, this research aimed to study the mechanisms of action and the effectiveness of these bioremediation agents. The research consisted of a bibliographical review, using database resources and the library collection of the University of Passo Fundo, totaling 56 materials (articles and books), after compilation and evaluation, it can be concluded that radiotrophic fungi are promising as bioremediation agents, with potential for protecting equipment, human beings and as biosensors to detect the presence of ionizing radiation.

Keywords: Radiotrophic fungi; Nuclear Waste; Bioremediation.

Resumo

Os resíduos nucleares, provenientes de combustíveis nucleares e acidentes nucleares, representam um grande risco ao meio ambiente e ao ser humano, causando diversos problemas como malformações, câncer, podem tornar áreas inabitáveis, causando alterações na fauna e flora de áreas inteiras. Na busca por uma forma mais eficaz de lidar com tais contaminantes, propõe-se a utilização de fungos radiotróficos, como os encontrados habitando o reator de Chernobyl na Ucrânia, nos polos e na estação espacial internacional, devido à sua alta resistência a esses contaminantes, seu poder de absorção de radiações ionizantes e a deposição de radioisótopos em suas paredes celulares, possibilitando a remoção dos mesmos do ambiente em que se encontram, bem como o consumo de matéria orgânica, como o grafite presente no reator número 4 de Chernobyl. Outra propriedade a ser explorada é o uso protetivo desses organismos para reduzir a incidência de radiações ionizantes em áreas de interesse e proteger os seres humanos. Portanto, esta pesquisa teve como objetivo estudar os mecanismos de ação e a eficácia desses agentes biorremediadores. A pesquisa consistiu em uma revisão bibliográfica, utilizando recursos de banco de dados e do acervo da biblioteca da Universidade de Passo Fundo, totalizando 56 materiais (artigos e livros), após compilação e avaliação, pode-se concluir que os fungos radiotróficos são promissores como agentes de biorremediação, com potencial para proteção de equipamentos, seres humanos e como biossensores para detectar a presença de radiação ionizante.

Palavras-chave: Fungos radiotróficos; Resíduos Nucleares; Biorremediação.

Resumen
Los residuos nucleares, provenientes del combustible nuclear y de accidentes nucleares, representan un gran riesgo para el medio ambiente y para los seres humanos, provocando diversos problemas como malformaciones, cáncer, pueden convertir áreas inhabitables, provocando cambios en la fauna y la flora de áreas enteras. En la busca de una forma más eficaz de lidiar con dichos contaminantes, se propone el uso de hongos radiotróficos, como los que se encuentran en el reactor de Chernobyl en la Ucrania, los polos y la estación espacial internacional, debido a su alta resistencia a estos contaminantes, su poder de absorción de radiaciones ionizantes y el depósito de radioisótopos en sus paredes celulares, posibilitando su eliminación del medio en que se encuentran, así como el consumo de materia orgánica, como el grafito presente en el reactor número 4 de Chernobyl. Otra propiedad a explorar es el uso protectivo de estos organismos para reducir la incidencia de radiaciones ionizantes en zonas de interés y proteger a los seres humanos. Por tanto, esta investigación tuvo como objetivo estudiar los mecanismos de acción y la eficacia de estos agentes biorremediadores. La investigación consistió en una revisión bibliográfica, utilizando recursos de bases de datos y del acervo de la biblioteca de la Universidad de Passo Fundo, totalizando 56 materiales (artículos y libros), luego de la recopilación y evaluación, se puede concluir que los hongos radiotróficos son prometedores como agentes de biorremediación, con potencial para proteger equipamientos, seres humanos y como biosensores para detectar la presencia de radiaciones ionizantes.
Palabras clave: Hongos radiotróficos; Residuos Nucleares; Biorremediación.

1. Introduction

Nuclear waste can come from different sources, such as nuclear fission reactors, hospital equipment, and research facilities (United States Nuclear Regulatory Commission [U.S.NRC], n.d.). It causes great environmental impact, due to its power of nuclear emission, which brings diverse forms of damage, to the soil, fauna, and flora. Its long permanence in the environment aggravates that, with half-lives varying from days to millions of years, thus requiring long periods for its potentially toxic power to extinguish (Bourguignon & Scholz, 2016; Aquino & Aquino, 2012). This way causing areas impacted by accidents, such as Chernobyl's exclusion zone, to remain uninhabitable for extended periods, with estimates of at least 20,000 years (Auyezov & Balmforth, 2011).

Due to the increase in demand for clean energy sources, many countries are considering nuclear fission reactors, for their lower CO_2 emissions, and their potential to replace fossil energy sources, leading to cleaner energy production (International Energy Agency [IEA], 2019). This form of energy generation uses enriched uranium rods in order to generate electrical energy, which ends up generating nuclear waste (U.S.NRC, n.d.). This type of waste requires effective disposal, as there is currently no proven method for its safe long-term disposal (Nuclear Energy Agency [NEA], 2009).

These contaminants are commonly immobilized in materials like concrete, wrapped in a thick layer of steel and its subsequent underground deposit, in areas with low oxidizing power (Aquino & Vieira, 2002). However, this method, known as deep geological disposal, does not have proven efficiency, due to the extremely long periods—spanning millions of years—that these materials must be stored. Concerns regarding its safety have been raised, especially referring to accidents, and corrosion of the protective layers, which would lead to possible leakage of radioactive material. The uncertainties surrounding the long-term performance of this method of waste disposal should also be considered (NEA, 2009). These residues also have few forms of remediation, which are generally low in effectiveness, consisting basically of soil and water removal, as well as surface scraping, with the removal of these materials from the local (Onishi, 2014). Some measures were also taken in Chernobyl, with the use of large quantities of neutron-absorbing materials, such as boron compounds, to reduce the release of radionuclides. Lead, sand, and clay can also be used to absorb radiation, as was done during the Chernobyl reactor incident. (NEA, 2002).

Radiotrophic fungi were previously encountered in areas affected by high levels of ionizing radiation, like Chernobyl (1986). These fungi have a metabolizing potential for gamma rays and beta particles, with the absorption of these rays and particles favoring the growth of these fungal cultures. They have also demonstrated potential for retaining radioisotopes present on surfaces and in soil (Dadachova & Casadevall, 2008).

Considering this, the present review aims to study the use of radiotrophic fungi as bioremediation agents in areas contaminated with nuclear waste. To explore their capacity to assist in the safer storage of nuclear waste, providing a safer and more efficient way of disposal. Additionally, it seeks to examine their potential as protective agents, to reduce the incidence of ionizing radiation in areas of interest and to mitigate the damage caused to biological life forms.

2. Methodology

A qualitative, investigative research was carried out (Pereira *et al*., 2018), of the narrative literature review type (Rother, 2007; Mattos, 2015; Casarin *et al.*, 2020) which is not a systematic review. It is a simpler review with fewer criteria.

This study consisted of a bibliographic review, the research was conducted using materials such as books, articles, journals, etc. with materials available both physically and on the Internet (Moresi, 2003). The materials were collected using databases such as PubMed, Scielo, and Google Scholar, as well as materials available at the University of Passo Fundo.

3. Literature Review and Discussion

3.1 Nuclear radiation, effects on organisms and the environment

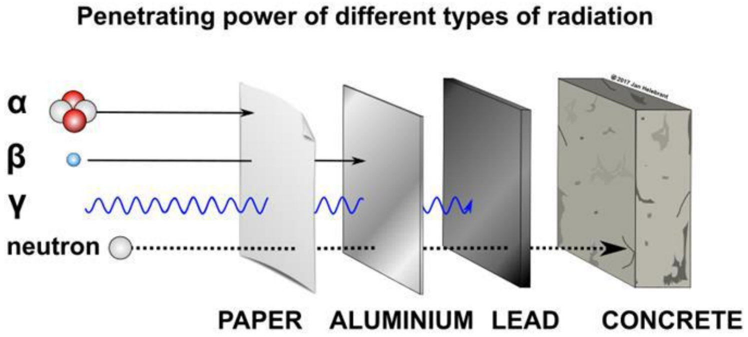
Radioactivity is the property of certain atoms that possess excess of energy or mass. These atoms generally have an excess of particles, which increases their mass, or an excess of energy, causing instability in the nucleus. These atoms by nature want to gain stability and they decay, emitting the excessive mass and energy in the form of alpha particles (α), beta particles (β), and gamma rays (γ), which is electromagnetic energy, having no mass. The excessive mass that is released in the form of alpha and beta particles makes the atom more stable, generally with greater stability if compared to its parent radioisotope. This process is called nuclear decay, mostly seen on isotopes, atoms of the same chemical element, with the same number of protons, and different number of neutrons, resulting in differences in stability between them. If the isotope is unstable and decays it is called a radioisotope, like the Cesium 137(^{137}Cs) (Fernandes, 2000; Atkins & Jones, 2010).

The types of radioactive emissions are:

- Alpha particles (α): Like a helium (He) atom without electrons, being made only by 2 protons and 2 neutrons, being this way a positively charged particle, represented by $^{4}\alpha_{2}$. Because it has high mass and charge it has low speed and penetration power, but can cause damage because of its electron deficiency, capturing them from the matter. Due to its low penetrating power, it can be blocked by the skin or a sheet of paper (Atkins & Jones, 2010).
- Beta particles (β): High-speed electrons, therefore a negatively charged particle, represented as β^-. Because it has less mass and higher speeds if compared to the alpha particles, it can penetrate up to 1 cm on the human body until it interacts with other atoms, it can be blocked by a 3 mm aluminum plate (Atkins & Jones, 2010).
- Gamma rays (γ): Refers to high-frequency electromagnetic radiation, being considered as high energy photons, having no mass, represented only as γ. It has the greatest penetrating power among the three forms of emission, it can pass through long distances, due to its high velocity, energy, and its highly ionizing power, it’s the one that causes more harm to life forms. To block it, thick layers of lead (Pb) sheets or concrete are necessary (Atkins & Jones, 2010).

The different penetrating powers of these emissions are represented in Figure 1 below:

Figure 1 - Penetrating power of ionizing radiations.



Source: Helebrant (2017).

These atomic emissions have ionizing properties, leading to the formation of ions, which are atoms with a lack or excess of electrons. Ions with an electron deficiency are called cations and have a positive charge (+). Otherwise, ions with an excess of electrons, having a negative charge (-) are called anions. These ions seek their stability by reacting with matter in their environment, altering chemical structures, potentially causing damage and mutations, with the formation of free radicals in organisms (Atkins & Jones, 2010).

Another complication about nuclear radiation is its long half-life, which is the period it takes for half of the initial atoms to decay, this time can vary between the type of atom and radioisotope in question. Having the potential to cause long-term damages, for example, Uranium (U) and its isotopes, which are used as nuclear fuel for electricity generation and in military vehicles, have a half-life that can vary, from 700 million years for the Uranium 235 (^{235}U) to 4.5 billion years for the Uranium 238 (^{238}U), demonstrating their long stay in activity (Aquino & Aquino, 2012).

The half-lives of some radioisotopes are presented in Table 1.

Table 1 – Half-lives of radioisotopes.

Radioisotope	Symbol	Half-life
Uranium 238	^{238}U	4.5 billion years
Uranium 235	^{235}U	700 million years
Cesium 137	^{137}Cs	30 years
Strontium 90	^{90}Sr	28 years
Cerium 144	^{144}Ce	290 days
Zirconium 95	^{95}Zr	65 days
Barium 140	^{140}Br	12.8 days
Iodine 131	^{131}I	8.1 days
Barium 137	^{137}Br	2.6 minutes

Source: Adapted from Aquino & Vieira (2002); Aquino & Aquino (2012).

This kind of waste is classified as dangerous and has limited options of reuse or recycling, as well as not many long-term storage ways. The most common way to dispose of these residues is the immobilization of them in concrete, bitumen or glass, being then stored in big steel drums, that provide shielding against the emitted gamma rays. These drums are later placed in large galleries or subterranean tunnels, with low humidity and corrosion potential, to minimize the damage that can be caused to the steel drums, leaving it viable for this use for longer periods, these galleries and tunnels are then sealed. Much is discussed about the safety and viability of this method, due to the long storage duration of these residues, and concerns about how the structure that holds these will behave over time. Few tests were conducted due to the long time these structures were designed to last (Aquino & Vieira, 2002; NEA, 2009).

The effects of exposure to nuclear radiation on human beings vary with time of exposure and dose. The symptoms shown in high doses, >1Sv (Sievert), include burns, tissue cellular death, especially the exposed ones, vomit, diarrhea and hair loss (Thomas & Symonds, 2016). Some people may also experience high fever, headaches, and severe damage to the DNA (UKEssays, 2018).

The long-term effects also called stochastic, occur without a threshold that is, there is no minimum dose, and the dose correlates with the probability of the effect, instead of proportional to the dose. Low doses can cause long-term damages, but most of the time these damages to the organism are healed by the cellular regeneration, avoiding major damages. Some of the stochastic effects include the increase in the risk of developing cancer, immunologic system deterioration, cardiovascular diseases, hereditary malformations, mutations, etc. It is a cumulative effect; the increased exposure to ionizing radiations, may lead to an increase in the probability of occurrence of the damages previously mentioned (Thomas & Symonds, 2016).

Regarding the environmental effects, the soil and plants are affected by high doses of nuclear radiation, and there is a risk of damage to the plant's tissues, growth inhibition, and mutations due to the damage to the DNA. The soil is also affected, making nutrient absorption more difficult, and potentially leading to infertility (UKEssays, 2018).

Strontium-90 (^{90}Sr), which emits beta particles and has a half-life of 28 years, forms highly soluble compounds that can be deposited in calcium-rich tissues, remaining fixed for years in bones and shells. The beta particles and the yttrium-90 (^{90}Y), a product of the ^{90}Sr decay, have radiotoxic potential by irradiating the bone marrow. Furthermore, ^{90}Sr and its isotopes, present in the environment can easily enter the food chain due to the high mobility of these compounds (Aquino & Aquino, 2012).

Water is considered one of the largest sources of uranium ingestion, being higher than food. Uranium (U) and its isotopes present in water sources, in the form of soluble salts, remain throughout the food chain for a long time, being absorbed by animals or humans that drink it and are later deposited on the bones and kidneys, replacing essential atoms to the organism and emitting radiation, which on the long-term proves to be harmful. The Radium (Ra) and Strontium (Sr) compounds, which are products of the Uranium decay, are highly soluble and easily enter the food chain and water. Due to their high similarity with Calcium (Ca), when absorbed by animals or humans, it is deposited on the bones and soft tissues (Aquino & Aquino, 2012).

With the dispersion of these atoms on the environment, they become part of the food chain, where they are deposited on plants, animal organisms, and water bodies, which are later consumed by other living beings. When consumed, these atoms are absorbed in their organisms, and successively in chain. This brings risks for those who consume it, and due to the long half-life of these atoms, they remain on the food chain for a long time (Aquino & Aquino, 2012).

Another environmental implication is the formation of the called fallout, a heterogeneous system composed of air, water, dust, soil particles, and radioactive atoms. It is a cloud and can be carried away to other regions by the wind influence. Its size, time of permanence, and movement depends on the meteorological conditions of the affected area. The fallout can emit ionizing radiation for months or years after its formation, and because it consists of a radionuclide-rich cloud, it ends up causing the dry deposition of these radionuclides from the air to the soil, as well as wet deposition in the form of rain. The speed of their deposition varies depending on the radionuclides present in the cloud and the type of soil in the area, with exposure to it being dangerous (Washington, 2014; Bourguignon & Scholz, 2016).

The most known nuclear accident happened in Chernobyl, Ukraine in the year 1986, occurred after a careless experiment with the cooling systems turned off, leading to an uncontrolled reaction and the subsequent reactor explosion, scattering radioisotopes around the area, and leading to the formation of the fallout that was dragged by the wind to other areas, spreading even further the contamination. The nuclear fuel that was on the nuclear plant and other materials came in contact, creating some sort of extremely radioactive lava. This lava was later covered by a protective layer of solid lead that works as a form of shielding against the radiation emitted by this material. This event allowed a lot of discoveries related to radioactivity, one example is the discovery of fungi resistant to ionizing radiation and their interactions with it. (Zhdanova *et al.*, 2000).

The main radionuclides released in Chernobyl after the accident are listed in Table 2.

Table 2 – Main radionuclides in Chernobyl and their half-lives

Radionuclide	Half-life
Iodine 131 (^{131}I)	8.04 days
Cesium 137 (^{137}Cs)	30 years
Cesium 134 (^{134}Cs)	2.06 years
Strontium 90 (^{90}Sr)	29.12 years
Plutonium 241 (^{241}Pu)	14.4 years

Source: Adapted from Bourguignon & Scholz (2016).

The presence of radionuclides in the air increases its electrical conductivity, after the explosion of Chernobyl's reactor number 4 in 1986, an increase of up to 10 times in electrical conductivity of the air was detected in regions like Helsinki, Finland, about 1300 km away from the reactor (Paatero, *et al.*, 2010).

Another effect that can be seen is that nuclear radiation can cause harm and even change the biodiversity of affected areas, causing mutations in animals and plants, one example is the Red Forest surrounding the nuclear reactor of Chernobyl. Due to the impacts of the accident, with damages to the soil and plants, the forest would be unable to stay alive, but even after years due to natural selection, more resistant plants, animals, and microorganisms started to inhabit the area (UKEssays, 2018).

In the exclusion zone there was an increase in the death of coniferous trees, invertebrates, and mammals. Reproductive dysfunctions and genetic anomalies in both animals and plants, caused by the radiation exposure were noticed, it also reduced the rate of biomass decomposition, negatively affecting plant growth in subsequent years (Bourguignon & Scholz, 2016).

3.2 Radiotrophic fungi

Fungi are organisms that belong to the fungi kingdom, they are eukaryotic, having their DNA surrounded by cellular membrane, they can be unicellular or multicellular, covering yeasts, molds, and mushrooms. The most common form of fungi are molds, they form large masses called mycelium composed by long filaments, called hyphae, that branches and spreads, similarly to roots on a plant. They are chemoheterotrophic beings that feed by absorbing and decomposing organic matter, releasing carbon dioxide ($CO_{2(g)}$) into the atmosphere. They are extremely resilient living beings that can use diverse sources of organic matter and energy to survive, and possess specific characteristics that allow their survivability in inhospitable environments (Tortora *et al*., 2019).

Radiotrophic fungi are microorganisms that were discovered, identified, and isolated in the year 1991. They were found growing around reactor number 4 of Chernobyl and in the Red Forest, their capacity of organic matter decomposition was noticed, since they were found decomposing the radioactive graphite from the reactor. In the next years over 2,000 strains, from 200 species, from 98 genera were discovered and isolated (bioprospected) in the areas surrounding the reactor (Zhdanova, *et al.*, 2004).

The sampling was conducted with the use of swabs on areas of fungal growth, samples were collected on 11 areas in total surrounding the reactor, with different levels of radiation. Some samples were incubated with antibiotics such as tetracycline, oxacillin, and ampicillin to inhibit bacterial growth, other samples were incubated without the use of antibiotics. In total 24 samples were collected, being then inoculated 2-3 days after the sampling, in malt agar, with the incubation occurring for 30-60 days in temperatures close to 25 ºC, after which the present species were analyzed. The main species detected were: *Cladosporium sphaerospermum, Penicillium hirsutum, Aspergillus versicolor, Aureobasidium pullulans, Penicillium expansum, Cryptococcus neoformans, Wangiella dermatitidis* and *Aspergillus niger* (Zhdanova, *et al*., 2000).

Examples of radiotrophic fungi extracted from Chernobyl are shown in Figures 2 and 3 below:

Figure 2 - strains of *Acremonium strictum* and *Aureobasidium pullulans.*

Figure 3 - strain of *Sydowia polyspora.*

Source: Zhdanova, *et al.* (2000).

The isolated fungi were analyzed and demonstrated in all radiation levels, a proportion of about 80% consisting of melanin-rich fungi, indicating the possibility of the correlation between melanin and the survival capability of these microorganisms. Some examples of melanized fungi found are: *Cladosporium sphaerospermum, Penicillium hirsutum, Aspergillus versicolor* and *Aureobasidium pullulans,* which were frequently seen during the 18 months of sampling. The analysis of the encountered species showed the predominance of *C. sphaerospermum* in the explored areas (Dadachova & Casadevall, 2008; Zhdanova *et al.*, 2000).

These melanized fungi are also called “black fungi” due to their coloration, usually darker than other fungi, since it has large amounts of melanin in their cell walls, which leads to changes in the color of the fungi and its hyphae, with these colors varying of shades of brown, gray and black, but can also exhibit lighter colors in more isolated cases (Chowdhary *et al*., 2014).

The exposure of these melanin-rich fungi to ionizing radiation, especially beta particles and gamma rays, favors growth of these fungi and its hyphae in the direction of the radiation source in some strains studied, this property was denominated radiotropism. Demonstrating these microorganisms’ ability to detect the presence of ionizing radiation, and to interact with it, favoring the growth and dissemination of these cultures. Another observed factor relates to their capacity to decompose carbon structures containing radioisotopes, such as the graphite from the reactor (Zhdanova *et al.*, 2004).

Experiments were conducted with the same fungi species, one melanized and the other not. When exposed to radiation, the melanized species showed a greater increase in growth, demonstrating their adaptation to the extreme environments from where they were extracted, suggesting the important role of melanin on their adaptation and survivability (Dadachova & Casadevall, 2008).

Some authors also have found these cultures in several other areas, such as nuclear reactor cooling pools, the Russian space station Mir, and regions with high solar radiation, such as the poles and the Evolution Canyon in Israel. This demonstrates their ability to colonize environments inhospitable to many biological life forms (Dadachova & Casadevall, 2008).

Experiments were performed, where strains of *A. versicolor* and *P. expansum*, were exposed to conditions of open space, without any type of protection, being exposed to the vacuum of space and to solar radiation for 7 months. After the experiment was concluded, the changes were analyzed and compared to the control sample, it was observed on the exposed strains an increase in mitochondria and vacuoles, as well as the increase in the melanin layer and polysaccharides in the *P. expansum* sample (Novikova *et al.*, 2007, as cited in Dadachova & Casadevall, 2008).

Mitochondria are organelles located on the cytoplasm of eukaryotic cells, their main function is to act in cellular respiration and in the production of Adenosine Triphosphate (ATP), thus consisting of a cellular energy generator. The vacuoles in turn, are hollow organelles surrounded by membrane, with the main function of storing substances like water, proteins and sugars, as well as to promote stiffness to the cells (Tortora *et al.*, 2019).

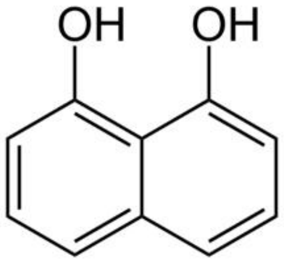
Observations made with melanin in these fungi, show that ionizing radiation modifies melanin properties, with changes on its electronic structure, evidenced by amplitude changes of the electron spin resonance (ESR) signal, changes on the electron transfer properties demonstrated a 4-fold increase in the reduction/oxidation of Nicotinamide Adenine Dinucleotide (NADH) in comparison to non-irradiated melanin. Analysis revealed the dependance of melanin on the growth of these substrates and pointed out a possible relation between melanin and the interactions with ionizing radiation (Dadachova *et al.*, 2007).

Melanin is a biochemical pigment with high molecular mass. It is typically black or brown in color and is formed through the oxidative polymerization of phenolic compounds. It is one of the most stable, insoluble, and resistant biochemical materials, this pigment is found in various living organisms. It can also be produced by fungi and serves several functions, such as shielding against ultraviolet (UV) rays and solar radiation, as well as acting as a pigment in organisms, such as human hair and skin. Melanin-rich organisms inhabit extreme environments, suggesting a connection between melanin and the resistance to these conditions (Dadachova *et al.*, 2008; Hill, 1992; Jacobson, 2000).

It can also be found in all the kingdoms of living organisms. Some of its contributions include shielding against UV radiation, as well as protecting against oxidizing and ionizing agents, having an important role in the survival of fungi in extreme conditions. High concentrations of melanin in fungi sometimes demonstrate a role in the pathogenesis of these organisms. It is an amorphous polymer, synthesized in the melanosomes, organelles related to lysosomes. It can be produced by one of two synthetic pathways, some fungi do it by an endogenous substrate via a 1,8-dihydroxynaphthalene (DHN) intermediate. Otherwise, it can be synthesized from L-3,4-dihydroxyphenylalanine (L-DOPA). Melanin's chemical structure is not known, but microscopic studies point out that it forms granules that attach to the cellular walls of fungi (Eisenman & Casadevall, 2012).

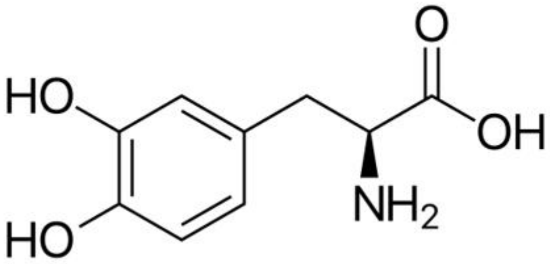
In Figures 4 and 5 below, the structural representations of 1,8-dihydroxynaphthalene (DHN) and L-3,4-dihydroxyphenylalanine (L-DOPA) are shown:

Figure 4 - 1,8-dihydroxynaphthalene (DHN).



(4)

Figure 5 - L-3,4-dihydroxyphenylalanine (L-DOPA).



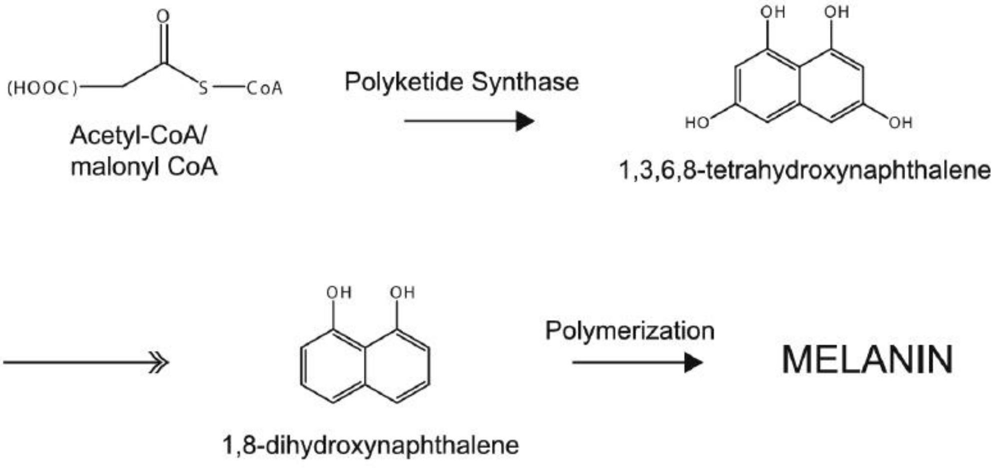
(5)

Source: Adapted from Wikimedia Commons (n.d.).

The first pathway mentioned, uses DHN as an intermediate, with Acetyl-CoA or Malonyl-CoA as precursors. These are produced endogenously, within the organism. In the first stage 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN) is formed, catalyzed by polyketide synthase (PKS). After the synthesis of 1,3,6,8-THN, it is then reduced to scytalone, with the later dehydration reaction that forms 1,3,8-trihydroxynaphthalene (THN). THN is reduced to vermelone, which is converted to 1,8-dihydroxynaphthalene (DHN) by dehydration. Finally, DHN is polymerized to DHN-melanin (Butler & Day, 1998; Langfelder *et al*., 2003).

The synthesis of melanin via the DHN pathway is presented in Figure 6 below:

Figure 6 - Melanin's synthesis via the DHN pathway.



Source: Eisenman & Casadevall (2012).

According to some experiments, due to melanin having a negative charge it can interact with various structures, tests showed the interaction between *A. niger* with *sepia* ink on water, providing the possibility to remove the ink from the water. Melanized lichens containing high concentrations of ionic uranium, iron, and copper associated with melanin were isolated from an abandoned mine. Bacterial pyomelanin containing uranium ions from an in situ treatment of contaminated soil. These tests demonstrate great potential in the use of these fungi as bioremediation agents, as they can bind toxins and contaminants from areas (Lamia & Neji, 2010; Purvis *et al.*, 2004; Turick *et al*., 2008, as cited in Eisenman & Casadevall, 2012).

Melanized fungi have thicker walls and denser external layers, that increases the structural resistance, it also has less porous cell walls if compared to non-melanized fungi. It can be considered as a resistance mechanism to substances by reducing the permeability of the cell wall, it can protect the fungi by restricting passage of substances that otherwise would be harmful to them, such as antifungals (Jacobson & Ikeda, 2005).

Experiments were conducted to confirm these observations, showing greater metabolic activity in irradiated fungi such as *Cryptococcus neoformans,* noticing the increased electron transfer capacity of ionized melanin as well as the growth stimulus of these fungi. Melanized species like *Wangiella dermatitidis* and *Cryptococcus neoformans,* showed significant growth increase when exposed to levels of radiation 500 times higher than the background radiation. The strain of *Cladosporium sphaerospermum* analyzed presented significant growth increase even in environments with few nutrients. These observations lead to the theory of the possibility of ionizing radiation absorption and its use as an energy source, called radiosynthesis, similarly to photosynthesis carried out by the aid of chlorophyll, absorbing light in plants (Dadachova *et al.,* 2007; Shunk *et al.*, 2020).

Melanin also appears to have significant radioprotective properties, non-melanized *C. neoformans* and *Histoplasma capsulatum* are highly radiation resistant, but the high presence of melanin further improved survival to high doses. The perception of these properties of melanin is the combination of physical shielding against ionizing radiation, and the ability of melanin to quench short-lived cytotoxic free radicals, which would be toxic to the cells, preventing cellular and DNA damages. This property is due to melanin having stable free radicals, that quench external free radicals that can cause damage to the fungi's structure. It was concluded that melanin's radioprotective properties are due to its chemical composition, free radicals quenching and its spherical spatial arrangement (Dadachova *et al.*, 2008; Enochs *et al.*, 1993).

A form of pyomelanin, recently synthesized and extracted from a modified strain of *A. niger*, demonstrated greater radioresistance, if compared with the wild strain of the same fungus. This extracted pyomelanin, showed great potential in shielding against UV-C doses compared to those of space environment. Although this pigment was only tested in liquid form, it has already shown great potential for bio-shielding, which can be used on materials and human beings, to protect them from potential damage, both in radioactive areas and in space conditions (Koch *et. al*, 2023)

The growth increase of these fungi not only occurs when directly irradiated, but also by being indirectly irradiated, as assessed in tests, with these fungi receiving the radiation present in the environment, stimulating their growth. This response to radiation from the environment, suggests the use of these fungi as a way to detect ionizing radiation in points of interest, acting as biological sensors (Malo *et al.*, 2020).

Another suggested use is in bioremediation, a biological method for treating contaminated areas. In situ bioremediation consists of soil and water treatment without considerable environmental changes, such as excavations and water pumping. It is based on the use of organisms and plants to decompose and immobilize contaminants in soil, water, and air, providing treatment for the affected area and allowing the return to normality. It incorporates various microbiological, chemical, geological, and engineering techniques (Jørgensen, 2007).

Studies show that these fungi absorb and accumulate radionuclides, mainly the ^{137}Cs. In addition to Cs they can also absorb and deposit radionuclides such as: ^{7}Be, ^{60}Co, ^{90}Sr, ^{95}Zr, ^{95}Nb, ^{100}Ag, ^{125}Sb, ^{144}Ce, ^{226}Ra and ^{238}U. Suggesting its use as a form of removal of these radionuclides from the soil, acting as bioremediation agents (Boháč *et al*., 1990; Haselwandter & Berreck, 1994; White & Gadd, 1990).

The form of absorption varies depending on the fungus in question, with potentially toxic ions being absorbed by biosorption into the cell walls or other extracellular structures. The main area of absorption of these atoms occurs in the cell walls, the absorption increases in the presence of carbonates. The mechanism in turn also varies according to the atom in question. In some cases, these atoms can also be absorbed by the cells through the cell membrane (White & Gadd, 1990).

Another process that can be used in bioremediation is called bioleaching, that is the process in which specific microorganisms extract inorganic metals from minerals, rocks, and soil, releasing these metals in their soluble form in an acidic solution, which can later be processed or properly disposed of (Saldaña *et. al,* 2023). Experiments were performed using rocks with high uranium content, and containing various other radioisotopes in diverse concentrations. These rocks were subjected to the bioleaching process of two strains of fungi, the *Aspergillus hollandicus* and *Penicillium citrinum.* The analysis revealed that *A. hollandicus* exhibited high bioleaching effectiveness with samples containing low uranium concentrations. In contrast, *P. citrinum,* demonstrated greater resistance to high uranium concentrations and also showed high efficiency in leaching uranium from the samples (Mohamed *et. al,* 2023).

These tests showed a leachability of up to 72%, which ends up resulting in the formation of the leach liquor, a liquid system containing these radioisotopes in soluble form and organic acids. This requires the removal of this liquid material from the environment and to properly dispose/process these radionuclides, this way removing these radionuclides from the affected environment. Some of the radioisotopes, were also absorbed by these fungi in question, but to a smaller degree if compared to the bioleaching percentages. The possible solution to properly remediate the area would be the application of the bioleaching process various times, to remove as many radioisotopes as possible from the environment (Mohamed *et. al,* 2023; Saldaña *et. al,* 2023).

To prove these fungi's capacity of radioprotection, experiments were done where *Cryptococcus neoformans'* ghosts, which are hollow particles that resembles the melanized cells they were extracted from, by acid digestion, maintaining both shape and dimensions. Materials like *sepia* melanin, charcoal and lead foil were exposed to 40kVp and 10 mA x-rays. In this experiment the shielding effect could be detected and quantified, with 100mg of *C. neoformans'* melanin having x-ray attenuation of 41%, compared to 100mg of lead foil's 81% (Dadachova *et al.*, 2008; Wang *et al.*, 1996).

Tests carried out in the International Space Station (ISS), demonstrated that the fungus *Cladosporium sphaerospermum* grew and survived in a zero-gravity environment. The experiments were conducted with a single petri dish exposed to radiation, half of it with the fungal culture in potato dextrose agar (PDA) culture medium, and the other half was left empty, with the incident radiation on these being measured using Geiger counters for each side of the petri dish. The results revealed that the side containing 1.7mm of fungus showed some decrease of at least 1.53% in incident radiation rates at the start of the experiment, to around 2.37% when the fungus was fully grown. This fungal culture also showed an increase of about 21% in growth when exposed to these conditions. Due to their attenuation properties, results suggest the potential application of these fungi as a protective agent against ionizing radiation. They could be used to create fibers, which can be woven into fabrics for the manufacture of clothing and materials with radioprotective properties. These materials could be used in space travel, or as tarps to shield radiation-affected areas, among other uses. Additionally, they are promising due to their lightweight, when compared to steel, concrete, and lead, as well as for its capabilities to self-replicate and regenerate, for example (Shunk *et al.,* 2020; Pacelli *et al*., 2017).

Some studies also point out the possible use of fungal melanin internally, in cases of cancer to protect human organs, leaving tumors unprotected, providing a better way to deal with radiotherapy, making it more effective, as higher doses could be applied to the body without causing it harm (Schweitzer *et al.*, 2009).

These fungi decompose organic matter and can feed on practically any source of carbon, they do it to gain energy, grow, reproduce, and for cellular maintenance, proving easy to be cultivated even in areas with few available nutrients. It is also suggested its use in conjunction with other materials, as theorized, in situ available materials can be used to acquire better shielding effects, as 2.3 meters of radiotrophic fungi biomass, with approximately 7.2% melanin content, would be required to lower the incident radiation in Mars to levels equivalent to the Earth's. Whereas an equimolar system between these fungi and regolith, rocks found in Mars, would only be needed about 1 meter to achieve the same effects, lowering the incident radiation from 234 mSv/y found in Mars to 620 mrem/y or 6.2 mSv/y on average from Earth. Other factors such as saving fungal material can also be mentioned (Shunk *et al.*, 2020; Hassler *et al.*, 2014; U.S.NRC, 2021).

Other materials can be used to aid in this matter, such as Boron (B), which has a high capacity to absorb neutrons. Some boron compounds, such as borated polyethylene, are commercially available for radiation shielding. Due to its high hydrogen concentration, borated polyethylene can slow down incident radiations, lowering its energy and allowing the boron to absorb it effectively (Thibeault *et al.*, 2012).

4. Conclusion

The studies analyzed showed promising results in the areas relating to the use of these microorganisms as bioremediation agents, with success in capturing radionuclides from the soil, as protective agents reducing the incidence of radiations, being able to be used in the manufacture of materials, fabrics, and clothing, as well as in conjunction with other materials like Martian regolith, to reduce the ionizing radiation incidence in areas. It also shows potential to protect equipment and human beings, and to act as biosensors that can be used to detect the presence of ionizing radiation in places of interest.

It is a recent topic, driven by materials chemistry, space travel, and remediation of radioactive waste-affected areas. Studies and experiments must be conducted to further evaluate its use in places where nuclear waste is disposed of, and the potential to treat this waste more effectively than storing it underground.

Research both in controlled environments and in situ are required to evaluate process parameters, aiming to increase effectiveness. Additionally, the bioprospection and selection of strains with adequate characteristics and potential for use within the relevant legislations are extremely important.

Therefore, using cultivation techniques, and organic biomass in affected areas, could help the growth of these fungi, allowing them to absorb radionuclides available in the soil. Using other techniques in conjunction with the fungi bioremediation can also be beneficial, such as the use of other materials that can absorb ionizing radiation, like boron (B) compounds, which have capabilities in the absorption of radiations and neutrons, proving to be a good ally in bioremediation. Another important aspect is the ability to monitor variations in radiation levels, by observing the stimulus in the growth of these fungal cultures.

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