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RESEARCH ARTICLE

Prospects for fungal bioremediation of unburied waste packages from the Goiânia radiological accident

Ana Paula Gimenes Tessaro[1] · Leandro Goulart de Araujo[1,2] · Thalita Tieko Silva[1] · Ednei Coelho[3] · Benedito Corrêa[3] · Natalie Costa Rolindo[1] · Roberto Vicente[1]

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Abstract

Goiânia, the Goiás State capital, starred in 1987, where one of the largest radiological accidents in the world happened. A teletherapy machine was subtracted from a derelict radiotherapy clinic and disassembled by scavengers who distributed fragments of the 50 TBq ^{137}CsCl source among relatives and acquaintances, enchanted by the blue shine of the substance. During the 15 days before the accident was acknowledged, contaminated recycling materials were delivered to recycling factories in four cities in the state of São Paulo, Brazil, in the form of recycling paper bales. The contaminated bales were spotted, collected, and stored in fifty 1.6 m^3 steel boxes at the interim storage facility of the Nuclear and Energy Research Institute (IPEN). In 2017, a check of the content was performed in a few boxes and the presence of high moisture content was observed even though the bales were dry when conditioned and the packages were kept sealed since then. The main objective of this work was to report the fungi found in the radioactive waste after they evolved for 30 years in isolation inside the waste boxes and their role in the decay of the waste. Examination of the microbiome showed the presence of nematodes and fungal communities. The fungi species isolated were *Aspergillus quadricinctus*, *Fusarium oxysporum*, *Lecanicillium coprophilumi*, *Scedosporium boydii*, *Scytalidium lignicola*, *Xenoacremonium recifei*, and *Pleurostoma richardsiae*. These microorganisms showed a significant capacity to digest cellulose in our trials, which could be one of the ways they survive in such a harsh environment, reducing the volume of radioactive paper waste. These metabolic abilities give us a future perspective of using these fungi in biotechnology to remediate radioactively contaminated materials, particularly cellulose-based waste.

Keywords Goiânia accident · ^{137}Cs · Waste management · Radioactive waste · Fungi

Introduction

In the late 1980s, while the world was still sensitized by the Chernobyl accident, a radiological accident happened in Goiânia, Brazil, where scavengers looking for scrap metal to recycle took a ^{137}Cs teletherapy machine from a derelict radiotherapy clinic, disassembled it, ruptured the source, distributed small chunks of the radioactive salt among relatives and acquaintances, and released a fraction of the 50.9 TBq of the source into the environment, unleashing the biggest radiological accident in Brazil (IAEA 1988a).

The radioactive material spread into scrapyards, a paper recycling company, and the homes of many individuals. Four people died within a month after the opening of the source, and a total of about 4.5 thousand tons of radioactive waste were collected during the cleanup operation. The waste was conditioned in thousands of steel drums and boxes and disposed of in a repository especially built for it, in the city of Abadia de Goiás, approximately 20 km from the original contamination site (IAEA 1988b).

Only 15 days after the beginning of the cesium dispersion, the local health surveillance office acknowledged that many people were suffering from acute radiation syndrome and informed the authorities who proceeded on to

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✉ Leandro Goulart de Araujo
lgoulart@alumni.usp.br

[1] Instituto de Pesquisas Energéticas E Nucleares, IPEN/CNEN, Av. Prof. Lineu Prestes, 2242, São Paulo, SP 05508-000, Brazil

[2] Current Affiliation, Université de Lorraine, CNRS, 88000 Epinal, IJL, France

[3] Microbiology Department, University of Sao Paulo, Av. Professor Lineu Prestes, São Paulo 1374, Brazil

the appropriate treatment of victims and the identification and cleanup of the contaminated sites. Between the rupture of the source and the start of the response to the accident, contaminated metal scrap and recycling paper bales were sold and delivered to recycling factories in the cities of São Paulo, Osasco, Araras, and São Carlos, in the state of São Paulo, up to 1000 km from the original contamination site. The accident response team in São Paulo state collected the contaminated material from the factories and conditioned the paper bales in fifty 1.6 m^3 steel boxes, and the metal scrap in forty-three 200-L drums. This waste is presently stored in the interim storage of the Nuclear and Energy Research Institute (IPEN) in São Paulo city. Although maintenance was performed to fix the scratches on the paint and corrosion points, the packages remained without any other verification until the year 2017.

The interest in analyzing the waste came up in that year, aiming at considering some method of treatment of the paper bales to reduce the waste volume and to save some space in the storage shed, which was becoming full. This was a possible solution to the storage space problem as the delayed project of the Brazilian radioactive waste disposal site was progressing very slowly, with no prospect of starting receiving wastes in the next 5 years.

Two boxes were selected, and dose rates were measured to check recorded values and to assess the homogeneity of inside activity distribution. To open the boxes, a concern was that pressure could have evolved inside and blow a cloud of contaminated dust from inside as the lid was removed. When the paper bales were put in the boxes, they were dry and dusty, as can be seen in Fig. 1. After carefully shrouding one box in polyethylene sheet and creating a hood over the top covering, which was expected to avoid air contamination, seventeen screws, out of the twenty-four that fastened the lid against the rubber gasket and the box body, were removed. The remaining six screws in the row of one lid edge were only loosened to allow raising the lid and examining the content.

Surprisingly, instead of dust coming out of the box, what we saw was the bottom surface of the lid dripping water (Fig. 2). The pressure inside was apparently in equilibrium with the atmosphere as no air movement was

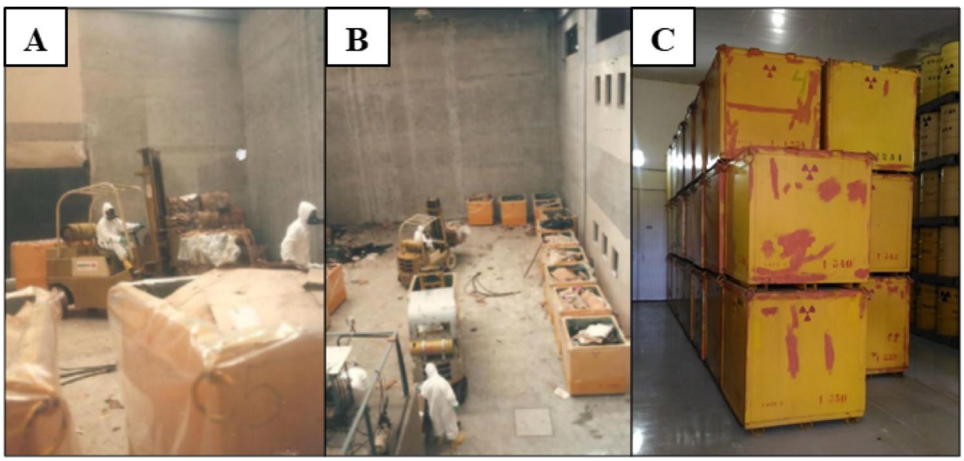

Fig. 1 Conditioning of the paper bales collected from ^{137}Cs contamination foci in São Paulo State (**A**, **B**). **C** Boxes with waste in the storage facility in Sao Paulo city. The stains are paint applied to fix corrosion points and scratches on the painting

Fig. 2 View of the inside of the box that shows the high moisture content

perceived when the screws were removed or the lid started to rise.

The paper bales that once filled the box to the top reached then not more than 70% of the height, and look like a black or dark brown cake. The color of the material, the cake-like appearance, and the presence of water were evidence of decomposition by microorganisms. The box was closed and plans were set to open the boxes, take samples to characterize the microbiome and the waste, and assess the treatment of the entire inventory of ^{137}Cs-contaminated paper bales by microorganisms to reduce the volume and eventually immobilize the waste in cement to prepare them for disposal. The sampling and analysis of the material had to wait 2 years and are now reported. Analyses of the samples under the microscope revealed the presence of nematodes, bacterial and fungal communities actively degrading cellulose.

This research aimed to isolate and identify the fungi, besides analyzing the ability of these microorganisms to degrade cellulose, thus a future perspective of using these organisms to assist in the treatment of paper-based nuclear waste. To the best of our knowledge, the identification of fungi in the radioactive waste inside sealed packages has never been investigated. Also, the drawbacks related to the possible formation of water in the waste of the Goiânia repository are discussed.

Materials and methods

This work is divided into three main sections: (i) measurements of the boxes containing cesium-contaminated radioactive wastes from the Goiania radiological accident by employing the dose-to-activity method (Tessaro et al. 2020); (ii) opening of the boxes, sample collection, and the identification of the fungi present in the waste; (iii) tests with the identified fungi on the degradation of cellulose.

Sample collection

There were two stages of sampling. In the first one, made in 2017, samples of 12 boxes were randomly selected from the 50 boxes in the storage. The boxes were weighed using the forklift scale, transported individually to a low background radiation area, out of the storage facility, and had their dose rates measured. The measurement of the dose rates was used to estimate the activity by the dose-to-activity method, using the MicroShield® v.9.03 software. Results of dose rate measurements at the distances of zero, 0.5, and 1.0 m from each of the four lateral sides of the package surfaces were used to reduce the uncertainties of the estimates. They were also used to model the distribution activity in each container to better correlate with the measured dose rates.

The results of the measurements were used as input to calculate the estimated activities. To take into account the large inhomogeneity of the radioactive content, the measurement of each side was attributed to 1/9 fraction of the waste mass, as the modeling considers a 3 by 3 matrix of homogeneous regions, and used the MicroShield® to refine the initial estimates (Fig. 3).

The procedure was repeated until an acceptable distribution of activity was obtained, which correlates with the measurements. The dosimeters used for the measurements were the Kromek RayMon10® radiation monitor and the Eberline FH 40F2 radiation monitor.

In the second stage of sampling, conducted in 2019, a further examination of the content and conditions of the boxes was undertaken. Samples with tens of grams were randomly taken from various spots on the surface or from a few centimeters deep of the partially decomposed paper cake using long-sleeved gloves attached to the polyethylene shroud, and conditioned in separate clean polyethylene containers. For the identification and measurement of ^{137}Cs, a high purity coaxial detector (HPGe) gamma spectrometer (Falcon 5000, Canberra) was used. The "In Situ Object Counting Systems" (ISOCS) software was employed to generate efficiency curves from the waste conditions, yielding the counting spectra. Counting was done for 60 min. The photopeak of 661.65 keV was used to measure ^{137}Cs activity concentrations. Waste sample moisture content was measured with a moisture analyzer, OHAUS, model MB200. Samples with approximately 10 g were kept at 100 °C until constant weight.

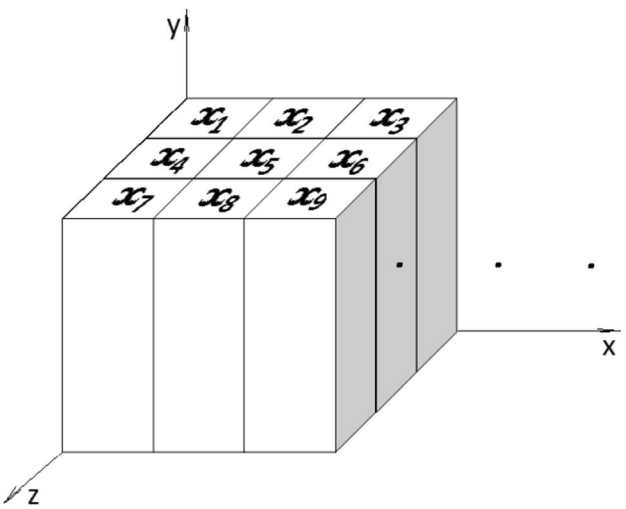

Fig. 3 Modeling of the MicroShield geometry (the dots are measurement points)

Isolation of fungi

To isolate fungi from the waste samples, 1 g of each sampling collection was mixed with 9 mL of sterile distilled water (10^{-1} dilution), followed by a series of dilutions up to 10^{-5}.

All dilutions were added in Petri dishes (90 mm × 15 mm) containing potato dextrose agar (PDA) culture medium. All analyses were done in triplicate. From each dilution, a 0.1-mL aliquot was removed and inoculation was performed on the surface of the solidified medium, by spreading the material using the streak plating technique. The plates were incubated at 25 °C for 10 days, following methods described elsewhere (Baquião et al. 2012; Assuncao et al. 2015).

Afterward, the quantification of the fungi was performed and expressed in colonies forming units per grams of starting culture (CFU g^{-1}) (Silva et al. 1995; Rice et al. 2012). To maintain the cellulolytic activity of the fungi, the isolated fungi were kept/grown in agar plates enriched with cellulose or carboxymethyl cellulose (CMC) as the only carbon source. The media were prepared with a mixture of (g L^{-1}) potassium chloride 0.5 g, magnesium sulfate 0.5 g, iron sulfate 0.01 g, agar 20 g, sodium nitrate 3.0 g, and cellulose or CMC 1% (Coronado-Ruiz et al. 2018).

Identification of isolated fungi—classical identification

The classical identification (macroscopic and microscopic) of the fungi was performed. For the macroscopic identification, the giant colony technique was used, inoculating 10 µL of the fungus suspension in the center of Petri dishes containing 20 mL of solid PDA media and incubating at 25 °C. After the growth, the characteristics of color, size, and texture of the colonies were observed. The microscopic identification was performed using the microculture technique proposed by Riddell (1950). In short, inside the Petri dish lined with filter paper, a glass slide was placed on a "U" glass rod. This set was sterilized in an autoclave at 121 °C for 15 min. A portion of the PDA medium (1 cm^2) was deposited on the slide. Using a nickel-chrome "L" handle, the fungi were sown around the culture medium and the filter paper moistened with sterile distilled water. The plate was kept in an incubator for 7 days at 25 °C after the microscopic characteristics of the fungi were analyzed. This procedure is aimed at assisting in fungal growth and the fixation of the fungi's vegetative and reproductive organs. The microscopic morphological structures of the fungi were then identified according to the literature (Barron and Peterson 1968; Von Arx 1970; Kozakiewicz 1989; Pitt and Hocking 2009; Seifert and Gams 2011). For examinations related to microbial life, the microscope Nikon, Eclipse E600, was used.

Extraction of the nuclear ribosomal DNA (rDNA)

The nuclear ribosomal DNA was extracted and purified directly from fungal colonies from cultivation on yeast extract sucrose (YES) agar (Abdollahi and Buchanan 1981; Degola et al. 2007), following the protocol of the PrepMan Ultra® kit (Applied Biosystems, Carlsbad, CA, USA). Therefore, the isolates were inoculated on YES agar and cultured for up to 7 days in an incubator at 25 °C. Then, a fragment of mycelium of approximately 0.5 cm^2 was cut from the periphery of the colony. The agar was removed and the mycelium was transferred to a 1.5-mL microtube containing 300 µL of PrepMan Ultra®. The microtube was vortexed for 1 min and placed in a water bath at 100 °C for 15 min and centrifuged for 7 min at 18,000 rcf at room temperature. The supernatant was transferred to another 1.5-mL microtube and stored at − 4° C for quantification and polymerase chain reaction (PCR).

DNA dilution

For quantification tests, the extracted DNA was diluted at 1:50 in Milli-Q water and quantified in NanoDrop™ 2000c (Thermo Scientific Waltham, MA, USA) at a wavelength of 260 nm. The absorbance was then measured at a wavelength of 280 nm to check for the presence and to calculate the purity of DNA using the ratio between optical density (OD) at 260 nm and 280 nm (OD260/OD280) (Sambrook et al. 1989). The DNA was diluted to 40 ng μL^{-1} for PCR.

Polymerase chain reaction

For PCR, 0.2-mL microtubes were used. The fragments of the ITS region of rDNA were amplified with primers ITS1 (5 ′TCC GTA GGT GAA CCT GCG 3′) and ITS4 (5 ′TCC TCC GCT TAT TGA TAT 3′) (White et al. 1990; Visagie et al. 2014). PCR was performed with 12.5 µL of PCR Master Mix (Promega, San Luis Obispo, CA, USA), 6.5 µL of Milli-Q water, 2 µL of DNA (40 ng), and 2 µL (20 pmol) of each primer (Prodimol Biotecnologia, MG, Brazil) to a final volume of 25 µL.

The amplification program included an initial denaturation (94 °C for 3 min), followed by 35 denaturation cycles (94 °C for 1 min), annealing at 57 °C for 1 min, and extension at 72 °C for 1 min (Visagie et al. 2014). At the end of the amplification, an extension step of 72 °C for 5 min was included.

After performing the PCR, all samples were subjected to agarose gel electrophoresis to verify the amplification efficiency. The agarose gel was prepared at a concentration of 1.2% agarose in a solution of 1 × TAE (Tris Acetate EDTA) plus Red Gel (Biotium, Hayward, CA, USA) or SYBR Safe (Invitrogen, Carlsbad, CA, USA) (1

µL for every 10 mL of gel). To apply the samples to the gel wells, 3 µL of the samples was previously stained with bromophenol blue (2 µL) and 3 µL was applied to each well. The gel was subjected to 100 V at 400 mA for 1 h. As a reference, to confirm the size of the fragments, 6 µL of 1000 bp Ladder DNA (Invitrogen) was added, prepared in 1:5 ratios (DNA Ladder:accompanying dye, v/v) in the first well of each row.

Purification of the PCR product

The purification of the product of the PCR reaction aims to remove primers, deoxynucleotides (dNTPs), enzymes, and salts from the PCR products that may interfere with the sequencing reaction. For that matter, the QIAquick PCR purification kit (Qiagen, Hilden, Germany) was used, following the manufacturer's instructions. After purification, PCR products were quantified and the purity of the purified PCR products was checked by NanoDrop™. For the sequencing step, only samples with purity values greater than or equal to 1.80 were used; otherwise, the PCR step was repeated (Baquião et al. 2012).

Sequencing reaction

The purified PCR products were sequenced bi-directionally (forward and reverse) using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The primers used were the same as those used in PCR. The reaction was performed with the following cycling: 25 cycles of 95 °C for 20 s, 50 °C for 15 s, and 60 °C for 60 s. After sequencing cycling, the product was precipitated to remove excess possible interferences. Just before the plate was placed in the sequencer, the fragments were denatured with 10 µL of Hi-Di formamide (Applied Biosystems) added to each well of the plate which was then sealed, and homogenized for 20 s in a vortex, followed by spinning and incubation at 94 °C for 3 min and thermal shock on ice for 5 min (White et al. 1990).

Electrophoretic separation

Capillary electrophoretic separation was performed by an ABI Prism 3100 sequencer (Applied Biosystems), using POP6 (Applied Biosystems) and 1 × TAE buffer for 54 °C for 30 min, 1300 V, with a 25% power laser. Consensus sequences were edited using the Sequencher program version 4.1.4 (Gene Codes Corporation, Ann Arbor, MI, USA). The sequences found were searched on BLASTn (www.ncbi.nlm.nih.gov) and MycoBank (http://www.mycobank.org/) (Altschul et al. 1990) to confirm preliminary identifications. The contig strings were deposited in the GenBank database.

Screening of enzymatic activity (cellulolytic)

The cellulose degradation by fungi was checked on plates. CMC was the only carbon source and was stained by Congo red. This dye reveals the activity of cellulolytic enzymes by binding itself to the polysaccharide substrate of the medium, forming transparent zones (yellow) around the colony (Yin et al. 2019). Five millimeters diameter mycelial disks of each fungus (Jo et al. 2009) was removed using a sterile cork borer and inoculated in the center of Petri dishes with CMC medium; the plates were kept in an oven at 25 °C for up to 10 days.

The experiments were carried out in triplicate. After incubation, the media plates were flooded with 1 g L^{-1} Congo red for 15–20 min. The dye was discarded, and the plates were flooded with 1 mol L^{-1} NaCl for 20 min. Finally, the NaCl solution was discarded.

Results and discussion

Opening the boxes and checking

After opening three boxes, the presence of high moisture content was confirmed (Fig. 2), as well as the marked reduction in the paper volume of the order of 10 to 30%. In 1988, the paper bales were filled up to the limit of the capacity of the boxes (Fig. 1). Therefore, the volume reduction is associated with moisture build-up, which could only be explained by microbial action.

The paper bales, originally tied and wrapped in a plastic bag, have fallen apart and the paper degraded up to the point that the cellulose fibers appear broken in the examination under the microscope. The paper in all samples appeared as small fragments, visibly degraded (Costa 2003), with colors varying from light brown to dark brown or black. In some parts of the box with the highest moisture content, it looked like a soft, wet mass, like moist clay.

Table 1 presents the results of the evaluation of dose rate differences between the measured and the calculated values. The values were based on the recorded activities of a sample of 14 waste boxes out of the fifty. The columns headed by "old" and "current" activities show the recorded activities for each box at the time of the conditioning of the waste and the calculated decayed present activity. The columns headed by "old" and "current," "measured," and "calculated" dose rates present the values obtained empirically in this work and those calculated with the recorded activities. It is visible that the differences between values of corresponding points

Table 1 Original and current waste box dose rates. The packaging date was from 03/01/1988 to 03/03/1988 (mm/dd/yyyy)

	Old	Current	Old dose rate		Current dose rate (measured)			Current dose rate (calculated)		
Box	Activity (MBq)	Activity (MBq)	0.0 m (mSv h^{-1})	1.0 m (mSv h^{-1})	0.0 m (mSv h^{-1})	0.5 m (mSv h^{-1})	1.0 m (mSv h^{-1})	0.0 m (mSv h^{-1})	0.5 m (mSv h^{-1})	1.0 m (mSv h^{-1})
261	3245	1619	200.0	13.0	43.1	16.8	8.3	93.4	38.8	18.0
339	810	404	50.0	3.0	9.7	7.4	6.9	22.7	9.5	4.4
348	1624	810	100.0	6.0	5.2	1.7	1.0	43.4	18.2	8.4
350	2272	1134	140.0	9.0	0.4	0.3	0.4	42.6	18.9	8.6
352	260	130	16.0	1.0	1.4	0.8	0.6	6.4	2.7	1.3
354	1624	810	100.0	6.0	4.0	1.5	1.0	43.6	18.3	8.5
1334	3245	1619	200.0	13.0	71.5	32.1	14.4	79.9	34.0	15.7
1336	714	356	44.0	3.0	0.4	0.4	0.5	18.8	7.9	3.7
1339	19,462	9711	1200.0	75.0	215.4	92.9	42.7	521.0	218.8	101.2
1340	648	323	40.0	3.0	1037.4	745.5	413.0	18.1	7.6	3.5
1346	1624	810	100.0	6.0	24.9	7.7	3.7	45.2	18.9	8.7
1356	1624	810	100.0	6.0	43.7	22.2	11.1	47.6	19.7	9.2
1357	324	162	20.0	1.0	2.5	1.9	1.5	9.5	4.0	1.8
1377	455	227	28.0	2.0	1.0	0.9	0.9	12.2	5.1	2.4

The measurements were performed on November 29 and 30, 2017

Table 2 Percent variation of measured and calculated dose rates and estimated activity concentration

Box	Recorded net weight (kg)	Measured net weight (kg)	Dose rate ratios				Current estimated activity concentration (Bq kg^{-1})
			On contact		At 1 m		
			Measured	Calculated	Measured	Calculated	
261	475	333	0.22	0.47	0.64	1.38	4.86×10^{6}
339	419	349	0.19	0.45	2.30	1.47	1.16×10^{6}
348	460	378	0.05	0.43	0.17	1.40	2.14×10^{6}
352	311	435	0.09	0.40	0.60	1.30	2.98×10^{5}
354	349	375	0.04	0.44	0.17	1.42	2.16×10^{6}
1336	350	388	0.01	0.43	0.17	1.23	9.18×10^{5}
1339	321	377	0.18	0.43	0.57	1.35	2.57×10^{7}
1340	372	349	25.94	0.45	137.67	1.17	9.26×10^{5}
1346	365	353	0.25	0.45	0.62	1.45	2.29×10^{6}
1356	430	322	0.44	0.48	1.85	1.53	2.51×10^{6}
1357	352	321	0.13	0.48	1.50	1.80	5.03×10^{5}
1377	300	377	0.04	0.44	0.45	1.20	6.02×10^{5}

are not negligible, confirming that the recorded activities may be different from the actual values.

Table 2 presents the variations obtained between the calculated and measured dose rates. The difference between these values was expected since the method used in the initial measurement in 1988 did not verify the four sides of the box in search of an average dose rate value. The relative variations are presented with the original measured values as reference for the calculation of variation.

A decrease in mass can be explained by loss of CO_2 formed in the decomposition of cellulose, to the environment, by escape through microscopic flaws in the sealing of the boxes. In the case of the mass increase, it is possible that there were registration errors at the time of the first measurements made in 1988. In a second round of collection, samples were collected and data related to humidity and pH were measured, as described in Table 3. Also, Table 3 shows the activity concentration of ^{137}Cs, which was measured in samples of these three boxes, using passive gamma spectrometry.

Measurement of cesium only in the fungi colony is impractical because it would require a huge amount of paper waste to be collected raising concerns of radiation exposure. Furthermore, the separation of sufficient amounts of fungi mass from the waste is extremely complicated for gamma counting, making the measurement unfeasible. Another issue is that cesium is not homogeneously spread for being a result of an accident, obviously not controlled, making it difficult for a comprehensive understanding of the radioactive cesium bioaccumulated and/or biosorbed in the microorganisms.

Examinations under a microscope showed that microbial life thrives in the waste mass. Figure 4 shows a sample stained with gentian violet (hexamethyl-p-rosaniline chloride) and confirms the presence of fungi.

Figure 5 shows a photogram, from a captured video of a living worm that was identified as a free-living nematode.

Nematodes are organisms with elongated tubular bodies (worms) and that inhabit the ground, hardly visible to the naked eye. Humidity and temperature above 28 °C favor the development of various types of nematodes (Basyoni and Rizk 2016). As a fact, we can assume that the Brazilian climate favors nematode development. Nematodes are fungi-interacting plant parasites that reside in the environment. These organisms can contain fungal spores in their internal structure, which increases their mobility and protects them from the harsh effects of the environment (Bergeson 1972).

Table 3 Results of moisture content, pH, and activity concentrations in the paper samples. Radionuclide: ^{137}Cs

Box no	Initial sample weight (g)	Final sample weight (g)	Moisture content (%)	pH	Sample weight (g)*	Activity concentration ($Bq\bullet kg^{-1}$)**
340	10.24	5.88	42.6	7	20.78	$(2.24\pm0.13)\times10^{4}$
350	10.38	5.22	49.7	6	37.12	19 ± 3
1334	9.78	5.02	48.7	7	21.19	$(2.67\pm0.13)\times10^{6}$

*Sample weight for the measurement of the activity concentrations; ** $\pm 1\ \sigma$ (68% confidence interval)

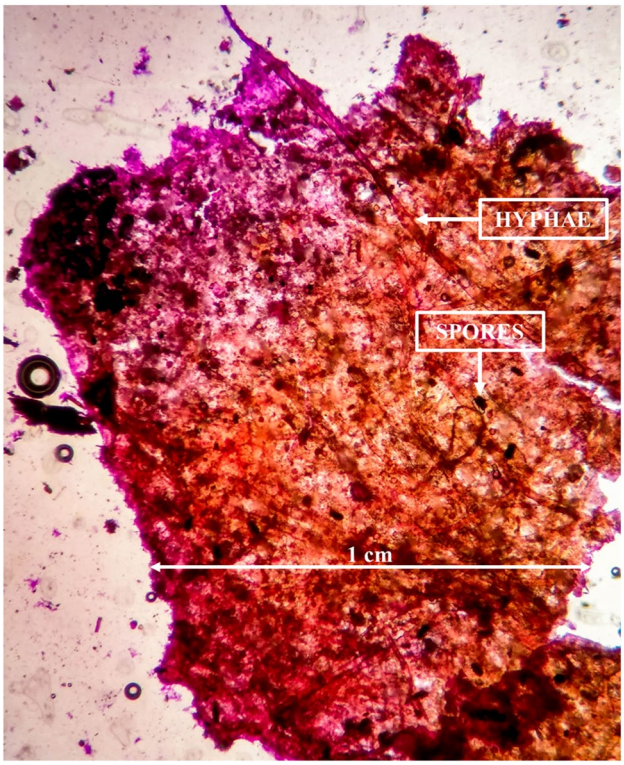

Fig. 4 Stained sample of degraded paper showing the original cellulose fibers and the microbial mass with the *hypha* and spores of *fungi*

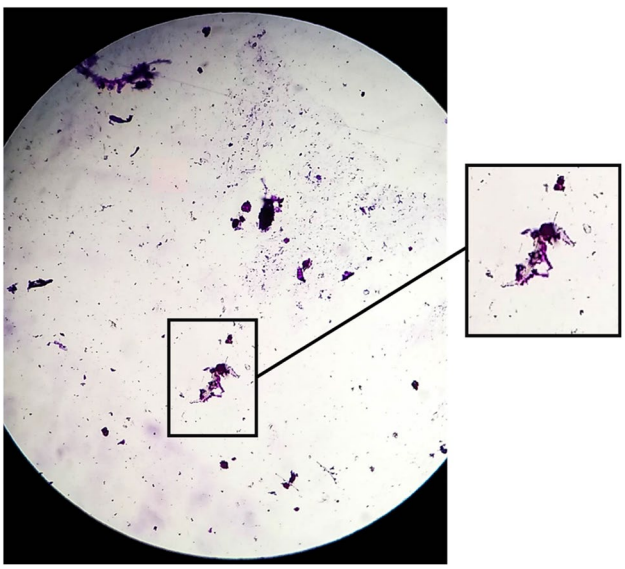

Fig. 5 A free-living nematode appears in this photogram of a video taken with a microscope from a stained sample of the paper mass

Nematodes appear to be a very radiation-resistant pest. The doses required for the non-hatching of eggs and mortality of these organisms are very high (Moy et al. 1999). Irradiation doses sufficient to cause acute mortality of parasitic nematodes will likely be impractical, as certain nematodes, such as *Meloidogyne javanica* and *Pratylenchus thornei*, may require radiation doses up to 7500 Gy (Myers et al. 2018).

According to studies conducted in Chernobyl, radioactive contamination can affect nematode communities directly or indirectly, altering their food resources and offering tolerance to particular species in a contaminated environment (selective pressure) (Lecomte-Pradines et al. 2014). The growth of nematodes and fungi in this type of radioactive waste has been documented for the first time. It is important to stress that the ^{137}Cs activity contained in even the most contaminated paper bales stored at IPEN can deliver an accumulated dose to the microorganisms that are lower than those that can affect survival of these species, except for very long-lived species.

Identification of isolated fungi

Seven species of ascomycetes of 7 genera were recovered from radioactive waste samples collected from the examined boxes. DNA sequence analysis of the rDNA genes of isolated fungi showed that they belong to the following species: *Aspergillus quadricinctus* (J.L. Yuill), *Fusarium oxysporum* (Schltdl), *Lecanicillium coprophilumi* (Zimmerman), *Scedosporium boydii* (Gilgado F.), *Scytalidium lignicola* (Pesante), *Xenoacremonium recifei* (Leão & Lôbo), and *Pleurostoma richardsiae* (Nannfeldt) (Figs. 6 and 7). More data on each species are given in Table 4.

Radiation may directly affect DNA in living organisms, ionizing the atoms in DNA molecules. On the other hand, radiation can also interact with other target atoms or molecules, most commonly water, causing indirect effect. Due to this, highly reactive free radicals are produced, which have the power to bind and damage targets like DNA (Desouky et al. 2015; Jeong and Jeong 2018). These effects can lead to numerous health issues in both humans and animals, as well as microbial cell death. However, some strains of fungi are able to withstand and adapt to such stresses (Jung et al. 2016; Okano et al. 2022).

Some of the fungus identified in our investigation produced pigments like melanin, which has been demonstrated in previous studies to increase fungal cell protection against radionuclides (Eisenman et al. 2020). The idea that fungi use melanin to harvest radiation to generate chemical energy has emerged from research on radiation resistance. The evidence for "radiosynthesis" is discussed elsewhere (Casadevall et al. 2017). Also, we hypothesize that these organisms were able to take advantage of the environment created in the sealed containers by using the material's cellulose as an energy source.

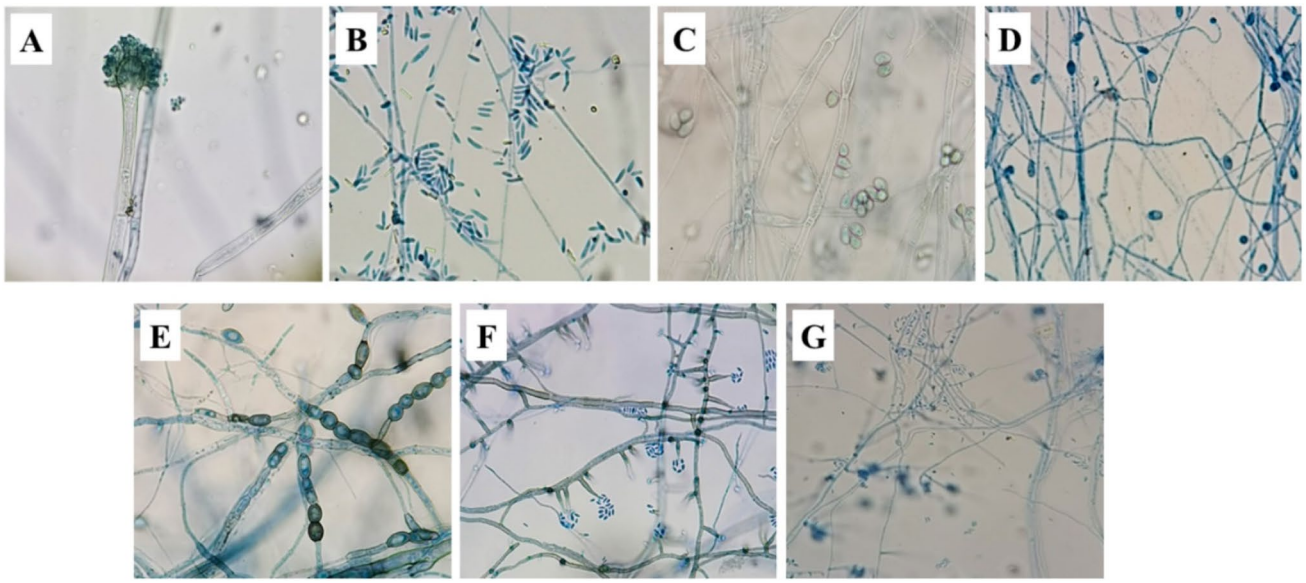

Fig. 6 Microscopic images of isolated fungi from samples of Goiânia radiological accident: **A** *A. quadricinctus*, **B** *F. oxysporum*, **C** *L. coprophilum*, **D** *Sce. boydii*, **E** *Scy. lignicola*, **F** *P. richardsiae*, **G** *X. recifei*

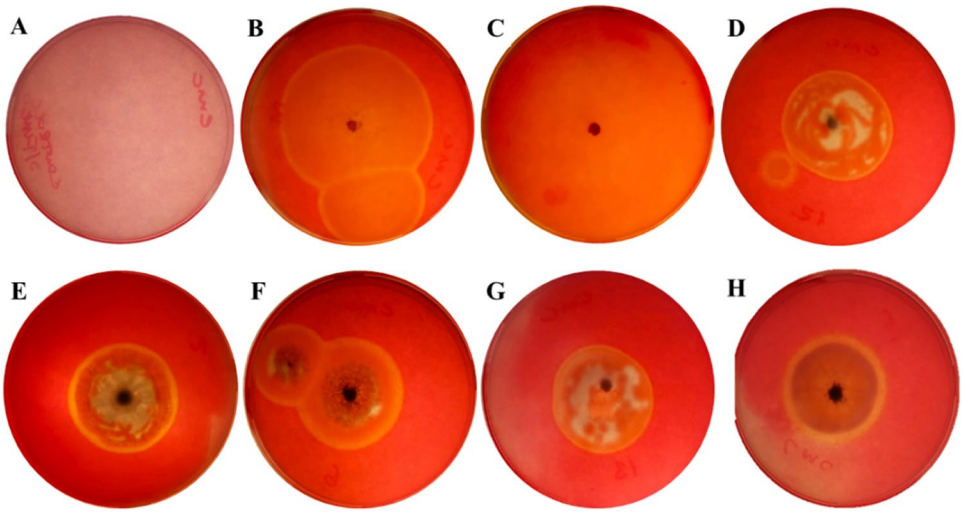

Fig. 7 **A**) Control, **B** *A. quadricinctus*, **C** *F. oxysporum*, **D** *L. coprophilum*, **E** *S. lignicola*, **F** *X. recifei*, **G** *S. boydii*, **H** *P. richardsiae*

Table 4 Isolates of fungi from cesium-contaminated radioactive wastes (isolation sources), collected in Brazil on October 7, 2019

Sequence_ID	Organism	GenBank
USPIPEN3	*Scytalidium lignicola*	MW136143
USPIPEN8	*Scedosporium boydii*	MW136144
USPIPEN9	*Xenoacremonium recifei*	MW136145
USPIPEN10	*Aspergillus quadricinctus*	MW136146
USPIPEN11	*Pleurostoma richardsiae*	MW136147
USPIPEN15	*Lecanicillium coprophilum*	MW136148
USPIPEN16	*Fusarium oxysporum*	MW136149

Increased reactive oxygen species (ROS) are produced when fungi are exposed to radiation. ROS can cause a number of oxidative alterations and disintegrative disorders in living organisms (Milojevic and Weckwerth 2020). Thus, genes linked to microbial tolerance are up-regulated, including those involved in DNA repair, cell defense, proteins and cell lipids, division, ribosome synthesis, and metabolism (Jung et al. 2016; Vasileiou and Summerer 2020). Also, fungi isolated from sites with high levels of ^{137}Cs showed an increase in spore germination which are exceptionally stress-resistant (Dighton et al. 2008).

Fungal strains exposed to extreme radiation conditions also showed carbohydrate metabolic over-representation, with enzymes involved (e.g., cellobiohydrolases, isocitrate, lyase AcuD) that allow the fungal to use alternative carbon sources with alterations in microorganism growth and differentiation (Blachowicz et al. 2019; Milojevic and Weckwerth 2020). Changes in carbohydrate metabolism are an adaptive response to stressful conditions (Blachowicz et al. 2019).

Table 5 shows a comparison between the fungi organisms found in this work and others contaminated by ^{137}Cs. Direct comparisons between these works are not only difficult given that paper contaminated by radionuclides is not commonly found in the literature but also due to the high concentrations of ^{137}Cs discovered in the paper bales. Nevertheless, Ohnuki et al. (2019) found ^{137}Cs activity concentrations in the same order of magnitude of our work (up to 10^6 Bq•kg^{-1}) by studying the migration of radioactive cesium in the Fukushima forest soil environment. This is a clear indication that some fungi organisms can be ^{137}Cs-tolerant even under different scenarios, providing that they have sufficient survival conditions, as those aforementioned.

The cellulose degradation by fungi was then studied by screening for enzymatic activity (cellulolytic). Figure 7 shows yellow circles around the colonies (hydrolysis circle) indicating cellulolytic enzymatic activity (Gohel et al. 2014; Yin et al. 2019). Seven fungi were used in this experiment: *A. quadricinctus*, *F. oxysporum*, *L. coprophilum*, *S. lignicola*, *X. recifei*, *S. boydii*, *P. richardsiae*.

A. quadricinctus can biosynthesize ferrichrome, which is a siderophore of the hydroxamate type (Hummel and Diekmann 1981; Diekmann 1984). Also, it can biosynthesize cellulase (Sethi and Rawla 1987), with the capacity to degrade rotten paper, which may explain the volume reduction of the paper bales. Until now, there is no report in the literature on the effects of radiation or radioresistance in this species.

F. oxysporum is a plant-pathogenic fungus that was found to cause vascular wilt diseases through the colonization of roots and stem, affecting the xylem vessel elements (Mace 2012), and in immune-compromised patients, in which it can produce life-threatening disseminated infections (Berman 2019). According to Tyupa et al. (2015), *F. oxysporum* presented resistance to uranium salts on activated sludge, in which silver was twenty times more toxic than uranium for this species. Recently, *F. oxysporum* was found in stained-glass windows in monuments in Belém do Pará (Brazil) (Pinto et al. 2019) and was able to biodegrade petroleum hydrocarbon in soil (Marchand et al. 2017).

L. coprophilum (Cordycipitaceae, Hypocreales) was recently discovered as a new species of fungus and was found in the feces of *Marmota monax* in China (Su et al. 2019). *Lecanicillium* W. Gams & Zare (Hypocreales, Ascomycota) is a paraphyletic and polyphyletic genus within the Cordycipitaceae (Sung et al. 2007; Su et al. 2019). There are also reports that this genus has members that parasitize varieties of arthropods, nematodes, and even other fungi (Goettel et al. 2008; Zare and Gams 2008). To the best of

Table 5 ^{137}Cs in fungi compared with the values obtained in this study

Organism	Origin	^{137}Cs activity concentration (Bq•kg^{-1})	Reference
Scytalidium lignicola	Cellulose	10–10^6	This work
Scedosporium boydii	Cellulose	10–10^6	This work
Xenoacremonium recifei	Cellulose	10–10^6	This work
Aspergillus quadricinctus	Cellulose	10–10^6	This work
Pleurostoma richardsiae	Cellulose	10–10^6	This work
Lecanicillium coprophilum	Cellulose	10–10^6	This work
Fusarium oxysporum	Cellulose	10–10^6	This work
Hygrocybe psittacina	Fruit bodies	10^5–10^6	(Ohnuki et al. 2019)
Russulaceae sp.	Fruit bodies	10^5–10^6	(Ohnuki et al. 2019)
Russula delica	Fruit bodies	10^4–10^5	(Ohnuki et al. 2019)
Lyophyllum sp.	Fruit bodies	10^4–10^5	(Ohnuki et al. 2019)
Truffle crops	Soil	1.78	(Mohammed et al. 2018)
Agaricaceae (mushroom)	Mostly fruiting bodies	2256.0 ± 690.00	(Mihok et al. 1989)
Boletus species	Edible mushrooms	3.3–22	(Falandysz et al. 2017)
Various species from the families: Boletaceae, Entolomataceae, Tricholomataceae, Russulaceae, Amanitaceae, Cortinariaceae, Strophariaceae, Cantharellaceae, Thelephoraceae, Polyporaceae, Hymenochaetaceae, Scutigeraceae, Ramariaceae, Gomphaceae	Mushrooms growing in soil	1.4–4100	(Fujii et al. 2014)
Wolfiporia cocos	Edible mushroom	< 1.4 to 12	(Falandysz et al. 2021)

our knowledge, this is the first time that *L. coprophilum* is found in paper bales.

S. boydii, in 2008, was proposed as a new species within the *Pseudallescheria/Scedosporium* complex (Gilgado et al. 2008) and, since then, has been frequently found in soil samples (Kaltseis et al. 2009; Sharma et al. 2013; Alvarez and Sanhueza 2016). Furthermore, it is related to skin human infections (Kaltseis et al. 2009) and the death of immunocompetent mice (Gilgado et al. 2009).

S. lignicola is an anamorphic ascomycete that has an omnipresent distribution (Büttner et al. 2018) and it is the causal agent of black root rot in several plants with economic value (Brito et al. 2020). The presence of this species may be related to dark-walled melanin, since the presence of the fungal melanin guarantees protection against radiological stressors, as found in samples from the Goiânia accident (Pacelli et al. 2017).

P. richardsiae (former *Phialophora richardsiae*) is a rare dematiaceous dark-walled fungus (Levenstadt et al. 2012). It was already found in a phaeomycotic cyst (Singh and Gumasta 2019), and it is associated with the decline of trees such as grapevine, olive, and neem (Carlucci et al. 2013; Ozben et al. 2017; Ghasemi-Sardareh and Mohammadi 2020; Lawrence et al. 2020). For the first time, *P. richardsiae* was found in radioactive waste.

X. recifei (Leão & Lôbo) L. Lombard & Crous (previously *Acremonium recifei*), besides being found in the samples from the radiological accident in Goiânia, was also identified in soil and Homo sapiens substrates in Brazil by phylogenetic analysis (Lombard et al. 2015). Nurunnabi et al. (2020) found many endophytic fungi, including *X. recifei*, in the plant *Sonneratia apetala* (Buch.-Ham) from the Sundarbans mangrove forest, Bangladesh. Purahong et al. (2019) also found *X. recifei* in the characterization of the *Castanopsis carlesii* deadwood mycobiome. Furthermore, this species was also reported to cause post-traumatic keratomycosis and isolated from skin diseases (Hurst 2019).

In 1997–1998, Zhdanova et al. (2000) isolated fungal species within a damaged power plant reactor in Chernobyl, and two fungal species, *Aspergillus* sp. and *F. oxysporum* species, were also reported in our work.

Fungi are known degraders of cellulose and produce a variety of cellulases. Endoglucanase, cellobiohydrolase, and β-glucosidases are enzymes that hydrolyze the β-1,4-glycosidic bond connecting D-glucose units. These enzyme assemblages are the principal used by fungi to degrade cellulose into glucose. Oxidative enzymes are also involved in cellulose degradation, such as lytic polysaccharide monooxygenases (LPMOs) (Morgenstern et al. 2014; Couturier et al. 2016).

Although enzyme analysis has shown the presence of fungi actively degrading cellulose, the degradation of cellulose waste contaminated with radionuclides by fungi can take many years to happen. The degradation of cellulose by microbial communities is considered a complex process since many enzymes are generated and produce carbon dioxide, hydrogen, and methane. According to Beaton et al. (2019), the complexity directly affects the span of the degradation by microorganisms.

The estimation of time in our study is especially challenging because of the unknown availability of microorganisms capable of degrading solid cellulose over time and the presence of significant amounts of radioactive cesium. There are estimations given in literature that vary from 100–500 years (Pavasars et al. 2003) to 100,00 to 10,000,000 years (Van Loon and Glaus 1997), which highly depend on the degradation conditions, such as pH—circumneutral to alkaline pH conditions are more suitable to this goal (Beaton et al. 2019). Most of the species isolated from the paper samples were soil and plant litter saprotrophs and *F. oxysporum* and *S. boydii* are plant pathogens.

Previously, some alternative approaches were considered for the volume reduction of the paper bales such as wet combustion in situ, which consists of adding an oxidizing reagent to the boxes' content, at room temperature, or the transformation of the contaminated paper into a pulp by dispersing it in hot water under agitation and filtration of the dissolved ^{137}Cs. The expected result would be a large volume reduction of the paper pulp, eventually reaching the unconditional clearance limit of solid waste. However, the biological degradation by fungi was chosen for being already started inside some boxes.

In the literature, fungus identification in radioactive waste is rare or non-existent. Individual experiments with each fungus are expected to be conducted in the future, to confirm their cesium resistance. The current research suggests that fungi could be useful in the remediation of radioactively contaminated materials, particularly cellulose-based waste.

Action of microorganisms in buried paper bales

Considering the mixed population of fungi identified, complete or partial degradation of cellulose can be achieved with the release of carbon dioxide and water under anaerobic conditions. Note that metal boxes have been sealed on the edges of their lid since the first management of the waste at the time of the accident, which prevented the entry of external moisture. In this condition, fungi were certainly already present in the contaminated environment and were able to develop under the harsh conditions mentioned inside the boxes. Eight random boxes were opened for checking moisture conditions and in all of them, we observed drops of water on the inside of the lid of the boxes. However, the amount observed varied from few to many drops, in addition to the varied amounts of moisture of the paper waste and reduction of the bulk volume. As shown in Fig. 8, in some

Fig. 8 Degraded dry paper waste from a random sample. The sample depicted is from Box 340 (see Table 3 for more information on this sample)

cases the paper waste is in an advanced stage of degradation and dryness, which indicates that microorganisms are little or not active anymore.

The volume of water possibly generated in the last decades in the buried paper bales in the Abadia-Goiás Repository raises concern. The Abadia de Goiás Repository, officially opened in June 1997, has the following characteristics: (1) underground concrete structure, filling material used to increase the rigidity of the internal structure and delay the migration of radionuclides to possible aquifers, ceiling barriers with layers of permeable and impermeable material; (2) distribution of packages considering the highest level of activity in the center and in the lower level of the structure; (3) filling the voids between the packages and the repository walls with a clay and sand compound with chemical and structural stability proven geologically and geochemically through tests.

Dealing with the safety of the final repository located in Abadia de Goiás, the project considered in its specifications the intrusion of water from rain; however, no consideration was given to the possibility of water accumulation inside the metal boxes, nor the possibility of internal corrosion of the same, despite reports related to the transport of radionuclides in the repository region. With the conditions observed in the opening of the boxes located at IPEN, it is possible to consider the possibility of leaching of ^{137}Cs into the soil and structural alteration when disposing of the packages.

Given the results, it is reasonable to assume that the hundreds of boxes containing contaminated paper bales that are in the Goiânia repository, as well as packages with radioactive waste that contain paper and other cellulose-based materials, are slowly decomposing by the action of microorganisms, either in intermediate storage facilities or in repositories. Depending on the local of the waste, the action of these microorganisms can be seen as advantageous or disadvantageous. On the one hand, the microorganisms detected can be used as a treatment process for the unburied paper bales, reducing the volume of the waste, concentrating activity, and facilitating disposal. On the other hand, the signs of internal moisture and corroding could be a topic of concern for the buried packages due to the generated volume of water that may facilitate the dispersion of cesium in the repository. Informal accounts report containers with cotton fabric filters once used in a monazite processing facility, and which are stored for 30 years in another storage facility, showing signs of internal moisture build-up to the point of corroding the containers and starting dripping.

Conclusion

The unexpected presence of high moisture content inside the waste boxes had two consequences: the first and immediate one was that the opening of the boxes did not require containment to prevent air contamination by spreading radioactive dust or microorganisms. Because of that, the containment hood was not further necessary.

Nematodes were visualized in the radioactive waste. Seven filamentous fungi species were identified and isolated from the boxes. The fungi survived the selective pressure, and for 30 years, they may have survived using cellulose as a carbon source. Also, due to having such metabolic capacities, the fungi may have helped in reducing the volume of paper waste.

The presence of microorganisms capable of degrading radioactive paper waste has its benefits but also drawbacks. They are seen as possible ways of reducing the volume of the waste, aiding its final disposal. Conversely, some concerns were raised on the possibility of corrosion, which may result in cesium dispersion in the repository.

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Author contribution APGT: conceptualization, data curation, funding acquisition, investigation, methodology, visualization; LGA: data curation, formal analysis, investigation, validation, visualization, writing – original draft; TTS: data curation, formal analysis, investigation, validation; EC: data curation, formal analysis, investigation, validation, writing – review and editing; BC: project administration, resources, supervision; NCR: data curation, visualization; RV: conceptualization, formal analysis, writing – review and editing.

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