doi.org/10.15392/2319-0612.2024.2762
2024, 12(4A) | 01-14 | e2762
Submitted: 2024-09-02
Accepted: 2024-11-21

Potential of Ionizing Radiation Application for Reducing Environmental Risks Related to Solids Residues

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Abstract: The enormous amount of solids residues and related cost for a suitable disposal have increased in megacities as São Paulo and Buenos Aires. Inovative technologies may be used in order to face managing progams with inovative techniques for for recycling or recover of materials. Gamma radiation was applied in laboratory scale for two different types of residues: fungi control in vehicular air filters, and for rat bedding waste (RBW) disinfection. Both materials may be used after irradiation and the radiation doses required will be discussed. Part of experiments carried out in Argentina evidenced the suitable use of vermicomposting for growing *Calendula officinalis*. Radiation doses from 10 kGy up to 30 kGy were tested for controlling microorganisms during the projects and were very effective. For filters disinfection experiments, DNA sequencing analysis for the fungi that survived after irradiation not only confirmed the presence of *Penicillium*, *Fusarium* and *Aspergillus* (toxigenic), fungi but also improved the data: the contamination included *Aspergillus niger*, *Aspergillus flavus*, *Penicillium glabrum* specie complex and *Fusarium incarnatum-equiseti* specie complex.

Keywords: *Aspergillus* sp, Gamma radiation, Biological residues (RBW), Vehicular filters.

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Potencial da radiação ionizante aplicada para redução de riscos ambientais relacionados aos resíduos sólidos

Resumo: A enorme quantidade de resíduos sólidos e os custos associados à sua disposição adequada tem crescido nas grandes cidades como São Paulo e Buenos Aires. Tecnologias inovadoras podem ser usadas no enfrentamento de programas de gerenciamento para a reciclagem e recuperação de matéria prima. A radiação gama foi aplicada em escala de laboratório para dois tipos diferentes de resíduos: no controle de fungos em filtros de ar de automóveis, e para a desinfecção de maravalha de biotério (RBW). Ambos os materiais podem ser reutilizados após a irradiação. As doses de radiação serão discutidas. Parte dos experimentos realizados na Argentina evidenciaram o uso adequado do produto obtido da compostagem para o cultivo da *Calendula officinalis*. Doses de radiação entre 10 kGy até 30 kGy foram empregadas para o controle de microrganismos durante os projetos sendo muito eficientes. Para os experimentos de desinfecção dos filtros, o sequenciamento do DNA de fungos que sobreviveram à irradiação não somente confirmou a presença dos fungos *Penicillium*, *Fusarium* and *Aspergillus* (toxigênico), como melhorou os dados da identificação que incluiram *Aspergillus niger*, *Aspergillus flavus*, *Penicillium glabrum* specie complex e o *Fusarium incarnatum-equiseti* (complexo).

Palavras-chave: *Aspergillus* sp, Radiação Gama, Resíduos biológicos (RBW), Filtros veiculares.

1. INTRODUCTION

Many urban centers face important challenges for managing suitable disposal of solid residues. Often these residues are deposited in landfills outside the region where produced. Health solids residues were 33.6 thousand tons/year (average - last four years) in São Paulo City, and recycled materials from houses were 90.3 thousand tons in 2023 [1]. In the Autonomous City of Buenos Aires, Argentina, 46% of the waste was disposed of in landfills and 54% was recycled [2]. Various types of residues offer biological contamination risks and as pointed out in food wastes, such as poultry litter, and other carcass residues may be source of pathogens in groundwater.

Millions of animals are still used globally for scientific research. Mice and rats constitute the majority of laboratory mammals employed in these studies [3]. This substantial use of animals generates significant amount of waste, including not only the animal remains but also the bedding material. The sheer volume of this waste raises serious environmental concerns regarding the impact of animal-based research. Bedding waste consists primarily of organic material and water, but it also contains bacteria, viruses, fungi, and parasites that can pose health risks to both animals and humans [4]. Proper management and control of animal waste are essential to prevent environmental pollution. Of particular concern are the wastes generated from facilities where animals are used for experimental purposes, and classified as hazardous.

Another important material that may be reused after disinfection may be the vehicular air filters. Substantial amount of them could be used twice. Cellulose filter media are commonly used in inlet air filters in motor vehicles and they are made of fibers with relatively large diameters, usually over 10 microns. New materials and combinations have been proposed for next air filter generation. To highlight the importance of cleaning and reuse such type of filters there is an estimative that more than 19 million of cars are circulating in São Paulo State and 6.2 million are in São Paulo the Capital, excluding taxi [5, 6].

This study examines data from Argentina and Brazil, focusing on the use of gamma rays from ^{60}Co sources for materials irradiation. The research aims to evaluate the efficacy of radiation in reducing microorganisms in vehicular filters (fungi), thereby enabling their reuse, as well as in treating biological residues from animal facilities to eliminate pathogenic microorganisms (bacteria and fungi) and produce a soil amendment. The hazardous microorganisms present in such waste must be effectively eliminated before reuse or disposal.

The key parameters for effective radiation processing of materials include radiation dose, dose rate, and the type and level of contamination. Not only DNA of microorganism is a target of irradiation but also many important structures of cells may lose their functions after irradiation. This radiation technology is widely used for the sterilization of medical products and in recent decades, ionizing radiation has demonstrated significant efficacy in mitigating organic and biological pollutants in wastewater and effluents. However, there remains substantial work to be done in addressing the decontamination of solid residues.

2. MATERIALS AND METHODS

Radiation gamma from Cobalt – 60 have been applied for reducing microorganisms from Rat bedding waste ***(RBW)*** in Argentina and from vehicular air filters in Brazil. A Multipurpose ^{60}Co Irradiator was used for filter irradiated with material protect with double paper envelopes. The dose rate was 5.5 kGy/h for experiments carried out in Brazil. In Argentina an 820 kCi – activity cobalt-60 source was used during irradiation and materials protected in polyethylene bags, with a dose rate of 20 kGy/h , and dosimetry was carried out with alanine dosimeters analyzed with an Electron Paramagnetic Resonance (EPR) system.

Experimental procedures carried out in Argentina comprised definition of biological specie for the experiment and they were related to the selected material (microorganism from the beddings waste; the *Eisenia fetida* earthworms for soil recovering and the *Calendula officinalis* seeds for plant growing experiments), as follows.

Rat bedding waste (RBW) was obtained from healthy laboratory rat species *Rattus rattus*, strain wistar and BDIX grow in captivity. The material was extracted from the cages after being in contact with the animals and feces for 3-5 days. *Wood shaving:* was used as a material for animal bedding. It was obtained from a by-product of pine (*Pinus* spp.) or non-resinous woods in timber industry. *Eisenia fetida:* was used in the vermicomposting tests. The earthworms were obtained from local organic compost producers. These annelids feed on decomposing vegetation and reproduce very quickly. *Calendula officinalis*: seeds were used in plant pot assay. They were commercially acquired from the selector "La Germinadora". It has the following indications: cultivation with a sowing depth of 0.5 cm, sowing time: summer, autumn and winter.

The RBW was characterized in its macroscopic composition, water content, pH, ammonium concentration, mesophilic aerobic bacteria (MAB), mesophilic anaerobic bacteria (MAnaB), aerobic and anaerobic spores (AS and AnaS), mycelial fungi, fecal coliforms, and *Escherichia coli*. Main media: peptone water, trypticase soy agar, TSA for plates agar; for fungi: potato dextrose agar and Sabouraud dextrose agar, incubated at 28°C for 5 days. The results were expressed as D_{10} values. All trials were conducted in at least two replicates. The confirmation of *E. coli* was carried out with EC Broth (OXOID) and incubated at 44.5 °C for 24h. The tubes that showed presence of microbial development and gas production were quantified and the results was expressed as most probable number (MPN)/g RBW of fecal coliforms [7]. D_{10} values for natural contamination in RBW were determined.

A count of microorganisms present in the vermicomposted material was made. The sample was serially diluted 1/10 in peptone water, and counts were made for mesophilic aerobic bacteria in trypticase soy agar (OXOID) using surface seeding and incubated at 32 °C for 48 h. The results were expressed as colony forming units (CFU)/g. *Vermicomposting test:* was performed in containers using Wood shaving; RBW + 10 kGy; and RBW + 30 kGy, and respective controls.

During the experimental procedures carried out in Brazil, aiming the disinfection of vehicular filters, the used filters samples collected in São Paulo were submitted to fungi determination before irradiations and fungi isolation was entirely based on inoculation in Petri dishes with swabs directly in Sabouraud agar and the incubation of samples during 7 days at 25ºC was under carefully standardized conditions, as described by [8].

Samples filters were collected in five districts of São Paulo. The samples were individually protected by an envelope and they were maintained in a box during irradiation, 15 kGy and 20 kGy were applied for fungal decontamination. From the irradiated samples part of them were submitted to DNA extraction, amplification and sequencing in order to confirm the fungi specie that survived after 15 kGy.

The radiorresistant strains (n=8) were confirmed by sequencing of the ITS, β-tubulina and calmodulin [9] gene regions of rDNA. DNA was extracted and purified directly from fungal colonies grown on yeast extract sucrose agar at 25 °C, in the dark, for 3 days using the PrepMan Ultra® kit (Applied Biosystems, Carlsbad, CA, USA). DNA was quantified with the GeneQuant pro Calculator (Amersham Pharmacia Biotech, Cambridge, UK). A fragment of ITS region was amplified with the ITS1 (5' TCCGTAGGTGAACCTGCG 3') and ITS4 (5' TCCGCTTATTGATAT 3') primer pairs. Part of the gene β-tubulin was amplified using primers T22 (5' TCTGGATGTTGTTGGGAATCC 3') and TUB-F (5' CTGTCCAACCCCTCTTAGGGCGACT 3'). Part of the calmodulin gene was amplified using primers CMD 42 (5' GGCCTTCTCCCTATTCGTAA 3') and CMD 637 (5' CTCGCGGATCATCTCATC 3').

After PCR, the products were purified with the QIAquick PCR Purification kit (Qiagen, Hilden, Germany) and stored at −20 °C until the time of sequencing. The PCR products were sequenced using the same primers as those employed for amplification. The Big Dye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) was used. The reactions were run on a 3100 DNA sequencer (Applied Biosystems). Consensus sequences were obtained using the AutoAssembler program (Perkin Elmer-Applied Biosystems) and SeqMan software (Lasergene, Madison, WI, USA). The sequences were used in BLASTn searches (www.ncbi.nlm.nih.gov) in order to confirm preliminary identifications. Figures 1 and 2 are showing type of samples submitted to microbial counting and irradiation.

Figure 1: *Rattus rattus* species from the wistar (A) and BDIX (B) strain, RBW (C) composed of wood shaving (D) and animal feces (E)

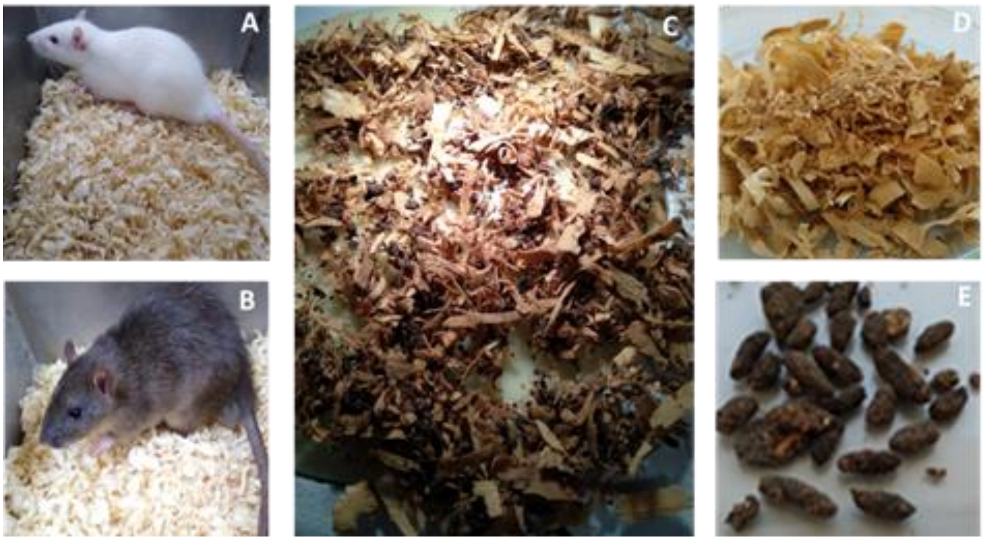
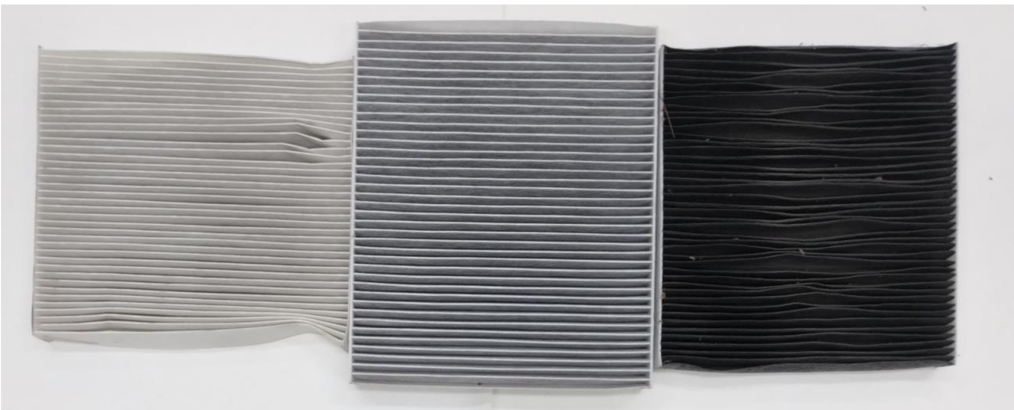


Figure 2: Vehicular paper filters (used) collected during sampling in São Paulo



3. RESULTS AND DISCUSSION

The challenges for a suitable management of solids residues have to be faced considering their impacts and risks to biota and environmental matrixes such as soil, water resources including groundwater. Integration among informal recycling sector is needed, same for advances in technology.

Radiation processing for microbial control is possible and few results showed in Table 1, D-10 values were presented. They mean the dose that inactivate 90% of microbial total count in rat bedding waste, in the experimental conditions. At Figures 3 it is possible to observe the changes in RBW materials in different periods and before and after irradiation (10 kGy and 30 kGy).

Progression of the vermicomposting process for wood shavings and irradiated RBW up to week 15. In week 1, the samples consisted of moist organic residues from fruits and vegetables. Largely disintegrated pieces were observed after week 3, the organic material becoming almost indistinguishable in the samples. Between weeks 4 and 5, earthworms were introduced to the substrates. They began circulating through the RBW within 3 days, while it took longer for them to do so in the wood shavings. By week 6, earthworms were observed moving throughout all substrates in the containers. By week 10, humus formation was visible on the surface, and earthworm cocoons were found within the material. At the end of week 15, the wood shavings remained, but the substrate - particularly the RBW - was enriched with a significant amount of earthworms and humus.

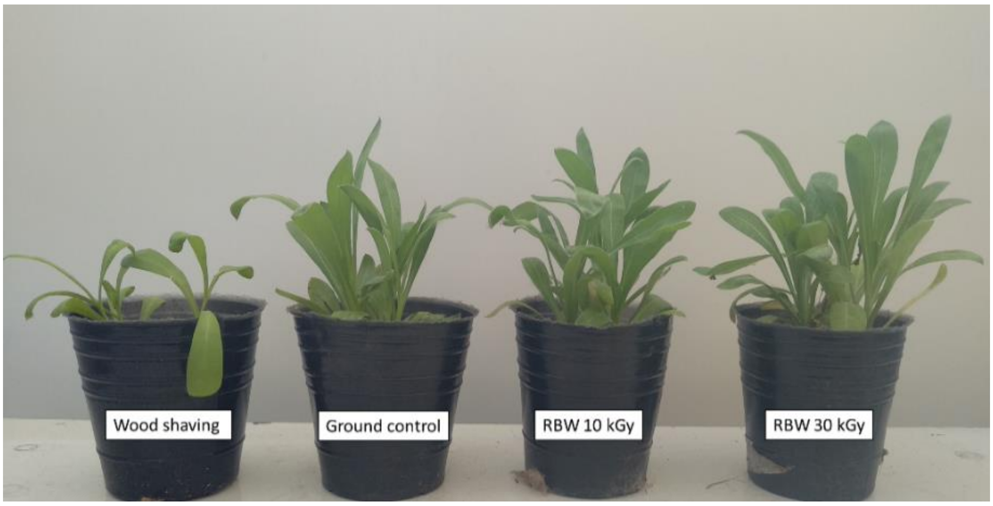
Table 1: D_{10} value for natural contamination in RBW

	D_{10}
MAB	0.62
MAnaB	1.0
AS	1.6
AnaS	1.7
Fecal coliforms	0.58
E. coli	0.68

Figure 3: Vermicomposting assay of wood shaving, RBW + 10 kGy and RBW +30 kGy over time

After 15 weeks, the worms were removed, and the material was used as an organic amendment mixed with soil and *Calendula officinalis* seeds were sown. Seeds germinated and after 21 weeks the plants grown with the RBW + 10 kGy substrates appear to have a growth, like the plants developed in the ground control (Figure 4). Remained healthy, presenting an abundant quantity of leaves and the formation of inflorescences was observed.

Figure 4: *Calendula officinalis* - 21 weeks old plants grown on different substrates



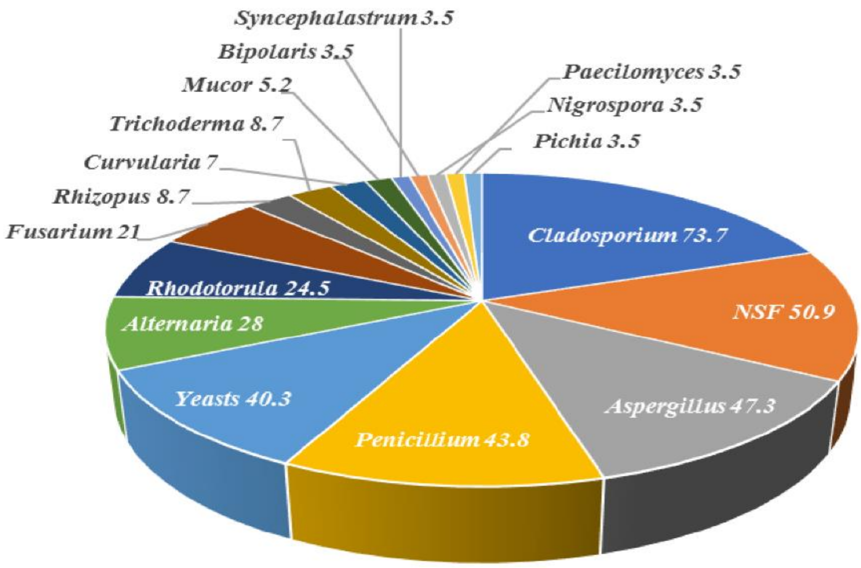
The previous figure illustrates plant development after 147 days. Seeds germinated and remained healthy across all substrates, with most treatments exhibiting abundant leaf growth and, by the end of the incubation period, the formation of inflorescences. Plants grown in substrates of soil-RBW + 10 kGy and soil-RBW + 30 kGy demonstrated growth comparable to those developed in the soil control. Conversely, seedlings cultivated with vermicompost wood shaving showed reduced foliar development.

This study highlights the potential of radiation technology to eliminate microorganisms in animal bedding waste (RBW) and transform it into a viable substrate for plant growth. The D_{10} was determined for the microbial groups present, indicating that in increasing order of sensitivity the fecal coliforms, *E. coli*, MAB, MAnaB, AS and AnaS were found. Laboratory animal husbandry facilities produce large volumes of hazardous material, primarily consisting of animal bedding waste, which contains potentially dangerous microorganisms that can transmit diseases to other animals and humans. Radiation can be employed to effectively eliminate these harmful microorganisms while facilitating the degradation of the waste components. The treated RBW can then be used as a substrate for vermicomposting, which, in turn, supports the germination and development of plant species such as *C. officinalis*. Thus, a hazardous material that would otherwise be incinerated can be converted into a recycled and valuable product through radiation technologies, contributing to more sustainable waste management practices.

Data obtained from the irradiated filters from São Paulo were reported in Figures 5, 6 and 7.

A total count of fungi was high and many specie were obtained. After irradiation treatment using 15 kGy most of them were completely eliminated.

Figure 5: Fungi diversity identified in the set of samples collected in urban areas of São Paulo



Several doses were applied for filter samples. Most of the fungi specie was sensitive to 10 kGy and 15 kGy (Figure 6). Nonetheless part of original contamination was observed in the irradiated samples. From those who survived 15kGy we applied other procedures for previous cleaning and irradiation and also the confirmation of specie was carried out with DNA techniques analysis.

More than 60 samples have been tested for fungi and irradiated showing a wide variety of fungi and further steps are planned for a safe reuse of vehicular filters [10].

Figure 6: Fungi diversity identified in the set of samples collected in urban areas of São Paulo

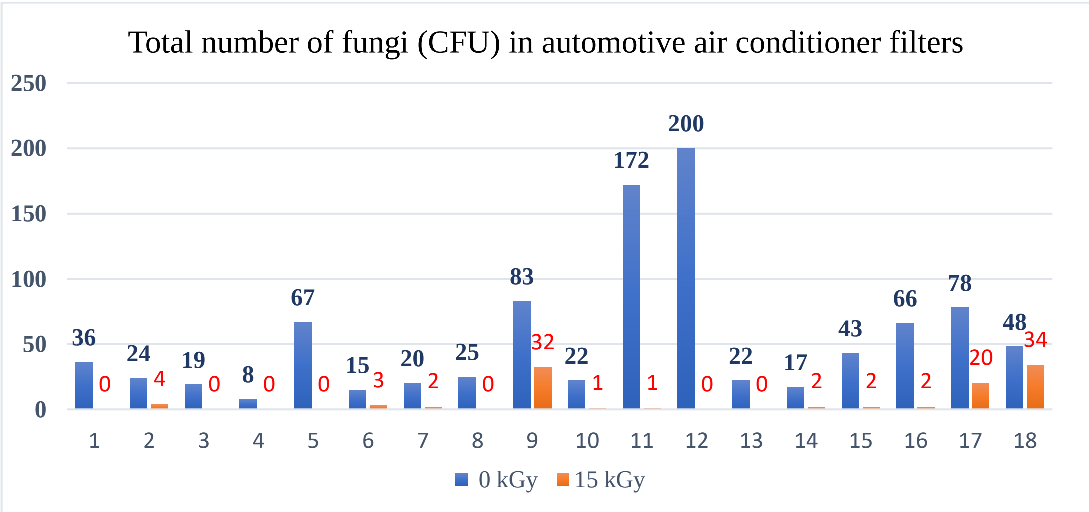
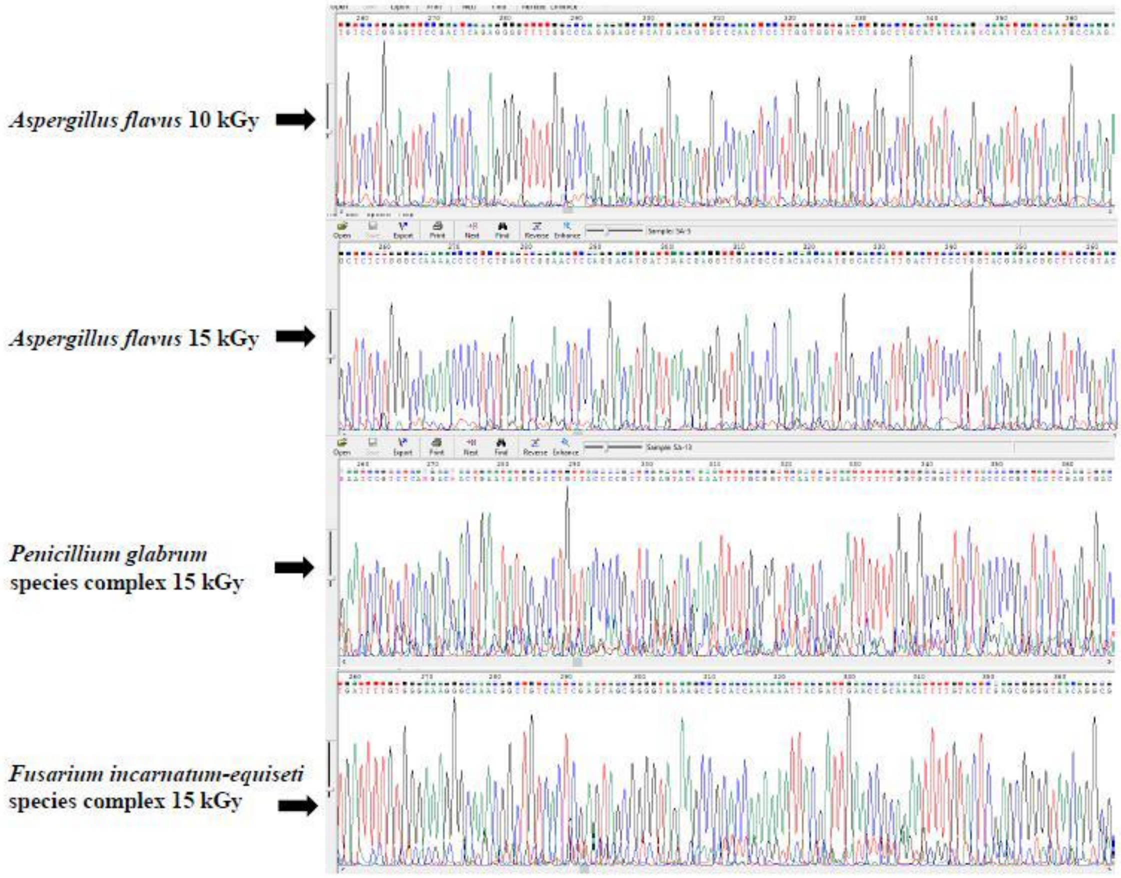


Figure 7: Radiation resistant fungi confirmed through biochemical methods (DNA Analysis).



4. CONCLUSIONS

As conclusion the authors may emphasize the needs for material reuse; technological advances and pollution control activities in a period with lots of evidences related to the climate changes, pandemic events, lack of disposal areas and impacts of flood, which again increase the amount of solids residues and human needs in distinct parts of the globe. Ionizing radiation may play an important role in this scenario.

ACKNOWLEDGMENT

This research was partially supported by IAEA and CAPES, IPEN and USP, who provided insight and expertise that greatly assisted the research. We thank Professor Benedito Correa, USP/SP for assistance with Fungi confirmation technique, DNA analysis, and also Tatiana Alves dos Reis, USP/SP.

FUNDING

International Atomic Energy Agency, IAEA, funding through the CRP Project (F 23033) and CAPES for providing student financial support.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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