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Full Length Research Paper

Bioaccumulation of cesium-137 and cobalt-60 from solid cellulosic-based radioactive waste simulates by *Plurotus pulmonarius*

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Solid cellulose-based radioactive wastes (CBW) constitutes in some cases, about 70% of the total solid low and intermediate level organic wastes originated from peaceful applications of nuclear technology in various fields of our life. Cesium-137 and cobalt-60 represent two of the most important radioisotopes spiking these waste categories. Both are serious contamination concerns due to their high energy gamma ray emitting (Cs-137 = 0.662 MeV and Co-60 = 1.17 and 1.33 MeV), besides ^{137}Cs is considered as one of long-lived isotopes ($T_{1/2}$ = 30.5 years). In this part of work, laboratory scale attempts were performed to follow bioaccumulation of Cs-137 and/or Co-60, found separately or together in a mixture of some solid CBW simulates. The process is based on the capability of *Pleurotus pulmonarius* to biodegrade the solid CBW simulates achieving acceptable weight reduction for the waste as well as reasonable bioaccumulation of the two isotopes from the spiked mixture, within their cells. Up to 134.95 and 41.1 kBq/kg (based on the dry weight of mushroom) were accumulated from Cs-137 and Co-60 respectively within a period of 54±3 days. It is worth mentioning that more than 54% weight reduction percent for the solid CBW simulates was acquired only due to a single cultivation process. Based on the data so far obtained, the bioremediation process for solid CBW based on the *P. pulmonarius* bioactivity seems to be simple, effective, and economical and can work where the other process cannot be applied.

Key words: Biological treatment, mushroom, *Pleurotus* spp., cellulosic waste, radiocesium, radiocobalt, gamma irradiation.

INTRODUCTION

The accumulation of solid radioactive wastes and the release of radiocontaminants followed by their subsequent dispersion in the environment are a subject of intense public concern. The major burden on the environment from radioactivity is due to discharged waste streams produced by industrial activities allied to the generation of nuclear power, in addition significant quantities of natural and artificial radionuclides were also released as a consequence of nuclear weapons testing, through accidental release, due to the peaceful applications of nuclear technologies in our life (e.g. medicine, research, industry, agriculture) and finally from the ongoing storage of nuclear materials massed over the past 60 years of nuclear activities (Lioyd and Renshow, 2005). Given the high costs and the technical limitations of current chemical – based approaches, there has been an unprecedented interest in the hope of developing cost effective bioremediation attitudes for processing of hazardous wastes (Lioyd, 2003; Agunbiade et al., 2009). Bioremediation is a natural process, and is therefore perceived by public as an acceptable waste treatment process. The operation based on capability of micro-organism to biodegraded the waste that accompanied by biostabilization of the contaminants within the living organism cells. The present work was designed to study the capability of *Pleurotus pulmonarius* to bioaccumulate Cs-137 and Co-60 during the biodegradation of spiked mixture of cellulose-based organic solid waste simulate. The distribution of both radionuclides between cap and stem of the mature fruiting bodies was followed. In

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Table 1. Elemental analysis of solid CBW mixture simulate.

Elements	Concentration (%)
Nitrogen	1.01
Carbon	32.4
Hydrogen	5.37

addition the weight reduction percent as well as biodegradation rate of the CBS were evaluated.

MATERIALS AND METHODS

Solid cellulose based waste (CBW) simulates

Equal weights of three categories of solid cellulose-based materials namely; cotton, paper and protective clothes in additions to polyethylene plastics were mixed together to form a mixture of the waste simulates that subjected to bioremediation process. The elemental analysis of the waste mixture simulate was determined by Flash EA series number 1112 and is represented in Table 1.

Radionuclides and chemicals

Radioactive cesium (Cs-137, $T_{1/2}$ = 30.5 years) and cobalt (Co-60, $T_{1/2}$ = 5.25 years) are most famous radionuclides found in numerous types of radioactive waste. Accordingly, they were used in this study to spike the cellulose – based solid waste simulate. The two radionuclides were purchased from Amersham life science company, England. All other chemicals and solvents used were of Analar grade and used without any further purification steps.

Pleurotus pulmonarius fungi

Biodegradation of the radioactive CBW accompanied with bioaccumulation and biostabilization of radiocontaminats was carried out using *P. pulmonarius* fungi (Samy cel3014, France). This strain was acquired from Agricultural Research Center, Giza, Egypt, as mycelia on malt agar medium 2 %w/v malt extract with 1.5% w/v agar.

Preparation of *P. pulmonarius* spawns

Wheat spawn was prepared according to Chang (1982). In 250 ml flask, 50 g wheat grains were added to one gram limestone chalk, suspended in 75 ml distilled water. After autoclaving, this flask was inoculated with mycelial disk (one cm in diameter) of *P. pulmonarius* and incubated at 26± 2°C for 15 days, after then the spawns were of well complete growth.

Irradiation of *P. pulmonarius* spawns

To enhance the capability of the microorganism for the biodegradation of solid waste simulates, at the end of incubation period, the spawns of *P. pulmonarius* were irradiated for 0.75 kGy doses in gamma radiation cobalt-60 cell at dose rate 1.56 Gy/min. The source of gamma irradiation used for irradiating the spawn of the fungus was cabalt-60 gamma cell 3500. This source is located at Middle Eastern Regional Radioisotopes Centre for the Arab Countries, Giza, Egypt.

Bioremediation of radioactive CBW

Three carrier free radioactive solutions, namely, one for ^{137}Cs, second for ^{60}Co and third is mixture of both radionuclides were prepared and used to spike the CBW substrates, separately. The specific activity of each solution was 165 Becqurel/ml. Ten sets each of 100 g oven dried CBW substrates were also prepared. Each of these CBW sets was enriched by with 4 g wheat brain and 4 g $CaCO_3$ then autoclaved. Four CBW sets were treated with ^{137}Cs solution, another two with ^{60}Co solution and the third two with the mixture of the both radionuclides. The last two sets of CBW substrates were not treated with any radionuclides and were used as control sets. Two sets spiked with ^{137}Cs were inoculated with the irradiated *P. pulmonarius* spawn. The rest spiked and also the unspiked (control) sets were inoculated with the non-irradiated spawn of *P. pulmonarius* following the method previously published (El-sayyad, 2008). Up to four harvestings were picked up from the various sets. Radionuclides biouptake by mushroom was analyzed by counting the contaminated ground fruiting bodies using Multichannel Analyzer PCA-A (Oxford instrument Inc, USA). Dry weight of the obtained mushroom was also measured. The weight reduction percentages of spiked and unspiked CBW sets were calculated according to Iljin et al. (1999) as follows:

$$\text{Weight reduction percent} = \frac{W_o - W_1}{W_o} \times 100$$

Where: W_o: is the initial weight of oven dried waste substrate (g), W_1: is the remaining oven dried residue of substrate in addition to the fruiting bodies of mushroom (3 harvests).

Also, biodegradation rate (g/day) was calculated according to the following equation:

$$\text{Biodegradation rate (g/day)} = \frac{W_o - W_1}{t}$$

Where: t is the time (day) taken up to the last harvest.

Photographs were taken to follow the growth of mushroom on the radioactive CBW substrate spiked with ^{137}Cs /or ^{60}Co and weight reduction effect.

Theoretical background

The ability of microorganisms including actinomycetes, cyanobacteria and other bacteria, algae, fungi, and yeast to bioaccumulate heavy metals and radionuclides from their external environment was investigated by many authors (Zajic and Chiu, 1972; Gadd and Griffiths, 1978; Brost- Pauwels, 1981; Shumate and Strandberg, 1985; Gadd, 1986a,b). Despite of the apparent simplicity of the biological technique for treatment of hazardous waste (that is, growing the microorganism on a media containing radioactive materials), yet to obtain reliable results within *in vivo* system is a big problem and more work is needed to establish this technique.

Studies that carried out by Francis (1998) and El-sayyad (2008) on the mechanisms of bioremediation of solid cellulosic waste simulates under various treatment conditions have been resulted in

Table 2. Bioaccumulation of Cs-137 from solid radioactive cellulose - based waste simulate by *P. pulmonarius**.

Flushing	Uptake (Bq)	Uptake (Bq/g**)	Uptake (%)	
			Stem	Cap
1st flushing	328.1	32.40	30.3	69.7
2nd flushing	68.6	26.49	30.0	70.0
3rd flushing	168.1	76.06	19.0	81.0
Total uptake	564.8	134.95	26.8	73.2

*Non-irradiated spawns were inoculated, **on the basis of dry weight of mushroom.

Table 3. Bioaccumulation of Co-60 from solid radioactive cellulose- based waste simulate by *P. pulmonarius**.

Flushing	Uptake (Bq)	Uptake (Bq/g**)	Uptake (%)	
			Stem	Cap
1st flushing	52.15	15.80	54.10	45.90
2nd flushing	43.40	4.48	51.10	48.80
3rd flushing	16.30	20.80	42.30	57.70
Total uptake	111.85	41.08	51.20	48.80

*Non-irradiated spawns were inoculated, ** on the basis dry weight of mushroom

the development of two operations:

1. Removal of the contaminants from the waste categories by the mushroom accompanied with biodegradation of the organic solid substrate and detectable reduction in waste volumes and weights was recorded.
2. Biostabilization of radionuclides and toxic materials compositions existing in the waste materials.

The biostabilization process of radionuclides found in the wastes is accomplished by exploiting the unique metabolic capabilities of microorganism. The radionuclides are biosolubilized by fungi directly through reductive dissolution enzymes or indirectly due to the production of organic acids metabolites during the biodegradation of the organic moiety of the wastes (Francis, 1998). The radionuclides released into the growing medium are biostabilized through the enzymatic reductive process e.g. bioprecipitation, biosorption and bioredistribution within the microorganism. The biouptake of radionuclides is claim to be depend on: the microorganism species, the surrounding environment and the physico- chemical properties of the substrates. For another point of view, metal biouptake in living system, may be energy dependent intercellular mechanism and may be a sequence of increased membrane permeability with a resultant exposure of further bind sites within the cell (Abdel- Hafez, 1999).

RESULTS AND DISCUSSION

The factors affecting the biouptake and biotransfer of radiocontaminants by mushroom are claimed to be: the concentration of total and available radionuclide in the wastes, pH-value and growing conditions in additions to the mushroom's trophic group e.g. biological family, genus and species (Kaduka et al., 2006).

CBW substrates were spiked with radioactive solution labelled with cesium-137 or cobalt-60 or mixture of both radionuclides. Mushroom spawns were inoculated on the spiked substrate. Up to four flushing were harvested periodically (36±4 days for the 1st harvest. The 2nd, 3rd and 4th harvests were picked up after 7±2, 15 and 21±2 days after the 1st harvest, respectively) and the radioactive contents in both stems and caps of the mature fungi were measured. At the end of the experiments the dry weights of the harvested fruiting bodies as well as of the remaining substrates were recorded.

The results presented in Table 2 illustrated the total biouptake of Cs-137 by *P. pulmonarius* from radioactive CBW spiked by radiocesium only. It is clear from the data obtained that:

1. The radiocesium biouptake at the first and third flushing is greater than that at the second one. It is worth mentioning, also, that the biouptake of Cs-137 at the end of the first flushing is still the greatest.
2. The radiocesium accumulated mainly in the cap of the fruiting bodies compared to that in the stem.
3. The total biouptake of Cs-137 at the end of the three flushing is 134.95 Bq/g (basis on the dry weight of fungi). By this figure *P. pulmonarius* shows high bioaccumulation capability for radiocesium relative to some other reported fungi. For example, according to Marten et al. (1996) the highest concentration of Cs-137 recorded in mushroom were 16.6 and 41.8 kBq/kg (based on organism dry weight) for *Lactarius* sp. and *Cortinarius* sp., respectively.

Similar trend was recorded for the biouptake of Co-60 form its radioactive CBW substrate (Table 3). The total

Table 4. Bioaccumulation of Cs-137&Co-60 from solid radioactive cellulose- based waste simulate by *P. pulmonarius**.

Flushing	Uptake (Bq)	Uptake (Bq/g *)	Uptake (%)	
			Stem	Cap
1st flushing	188.9	81.86	38.5	61.5
137Cs	136.3	59.26	23.5	48.7
60Co	52.6	22.8	15	12.9
2nd flushing	99.10	36.02	20.50	79.50
137Cs	87.80	31.90	15.70	72.90
60Co	11.30	4.10	0.80	0.60
Total uptake	288	117.88	32.20	67.70

*Non-irradiated spawns were inoculated, ** on the basis dry weight of mushroom.

biouptakes of Co-60 at each of three flushing were 15.8, 4.48 and 20.8 Bq/g, (basis on the dry weight of mushroom) respectively. However, it should be noted that the total biouptakes of Cs-137 was greater than that compared to that of Co-60 under the same growing conditions and during parallel harvesting periods. Also, it is clear from Table 3 that slight differences in the biodistribution of radiocobalt between the stem and cap in the mature mushroom was recorded. The data represented in Table 4 described the total uptake of Cs-137 and Co-60 from radioactive CBW, spiked with solution containing the two radionuclides, by *P. pulmonarius.*

At the end of the first flushing the total activity of 81.86 Bq/g bioaccumulated (based on the dry mass of *P. pulmonarius)* while was greater than that at end of the second period. It should be pointed that mushroom failed to produce any fruiting bodies after the second harvesting. It is clear, also, from the Table 4 that the biouptake of Cs-137 is usually greater than that of Co-60 even from substrate spiked with their mixture in the two harvesting periods.

In spite of that cesium-137 is long lived biotoxic radionuclides (Franta and Vanara, 1987). Yet, there were big differences in the bioremoval rates of both radiocontaminants (that is, Cs-137 and/or Co-60) from the spiked substrates under the same cultivation methodology and conditions. This may attributed to the high solubility of cesium compared to cobalt and hence it is easily filtrated to the organism cells. It should be also noted that, the bioaccumulation activities of the microorganisms decreased after the first flushing and they regenerated their abilities after the second harvesting again.

Hence, the third flushing characterized by high biouptake compared to the second. Hence reactivation the colonies of the fungi by inoculating fresh mushroom spawn periodically seems to be essential to keep the bioaccumulation process of the radionuclide in a continuous.

However, it is worth mentioning that the of both cesium and cobalt radionuclides could be detected up to the third harvesting within the period of 54±3 days. This could be supported by the proposal that mushrooms are characterized by acceptable resistance to environmental factors including metal toxicity and highly extreme irradiation conditions. Identical conclusion was reached by Fomina et al. (2006) and El-sayyad (2008).

Figure 1 illustrates the radioactive biodistribution in the stems and the caps of the fruiting bodies of mushroom through the successive flushing during the biodegradation of the CBW radioactive waste substrate. It is clear that Cs-137 was accumulated mainly in cap of *P. pulmonarius* and increase in the third flushing compared to the first and second ones (from≈70% up to more than 80% from substrate spiked with Cs-137 only). On contrary the radiocesium bioaccumulation in the stem was lower and decreased from the first two harvesting to the third one reaching 30.3% and down to 19.0%. On the other hand slightly differences between the accumulation of Co-60 in both stem and caps from substrate spiked with Co-60 only was recorded.

It is worth mentioning that the bioaccumulation of both Cs-137 and Co-60 from CBS substrate spiked with the two radionuclides increase in the caps in the second flushing to reach 79.50%. On contrary the bioaccumulation of both radionuclides in the stem drops from 38.5 to 20.5% at the end of the second flushing. Even so the bioaccumulation of Co-60 increased in caps with time while it decreased in cap simultaneously.

The variation in the biodistribution of Cs-137 and Co-60 between cap and stem of mature fruiting bodies referred to the behaviour of each radionuclide within the organism. Cesium is very soluble element and hence it smoothly transpired to cap and bioconcentrated there. On contrary, cobalt may be needed for some enzymatic activities in the organism e.g. alkaline phosphatase (Wolfe and Hoehamer, 2003). Also, according to Bilgrami and Verma (1974) it was found that fungi have the ability to synthesize vitamin B-12 (coblamins) and cobalt is

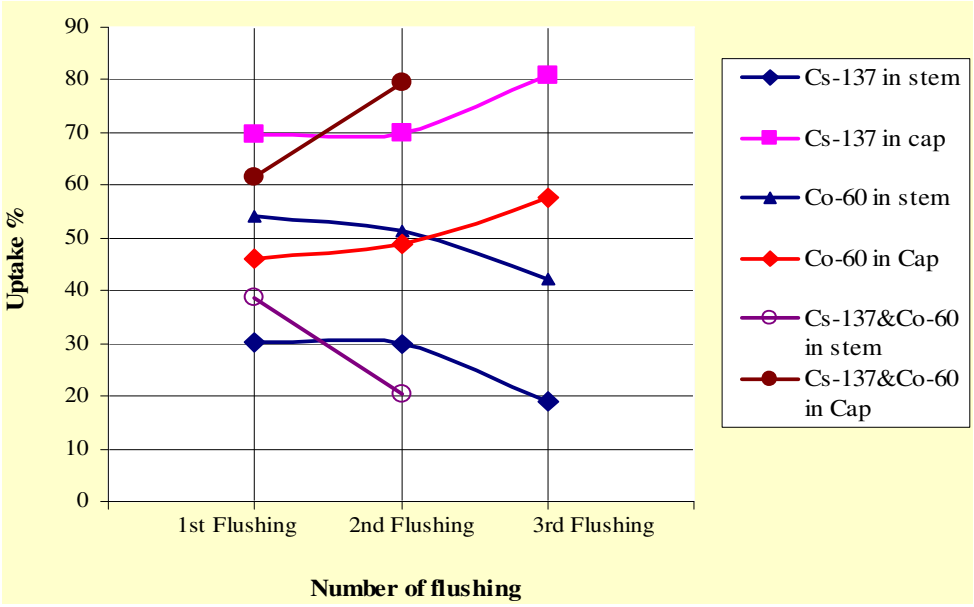


Figure 1. Bioaccumulation of cesium-137 and/or Co-60 by *P. pulmonarius* from radioactive cellulosic mixture waste simulate.

Table 5. Effect of irradiation pretreatment of the *P. pulmonarius* spawn on the bioaccumulation radicesium-137 from the soiled cellulosic mixture substrate spiked with radiocesium only.

Flushing	Bioaccumulation of cesium-137 Bq/g dry fruiting bodies	
	Non-irradiated	Irradiated spawn*
1st flushing		
Stem	24.5	47.5
Cap.	37.3	87.2
2nd flushing		
Stem	19.3	25.95
Cap.	31.6	40.9
3rd flushing		
Stem	30.6	43.5
Cap.	102.5	116.5
4th flushing		
Stem	-	93.04
Cap.	-	97.13

*Total irradiation dose was equal to 0.75 KGy.

known to be essential for this compounds. Therefore, it is nearly consumed by whole mushroom and hence there is slightly difference in its biodistrebution between cap and stem.

Table 5 described the effect of exposing the *P. pulmonarius* spawn to total irradiation dose equal to 0.75 KGy before its inoculation on cellulosic mixture substrate spiked with cesium-137. The bioaccumulation of radiocesium from that compost recorded slightly higher values compared to that of non-irradiated spawn. It is indeed quite striking to observe also that up to fourth flushing were harvested. This confirms again the earlier obtained results on the enhancement in the bioactivity of mushroom due to the irradiation treatment. However, it

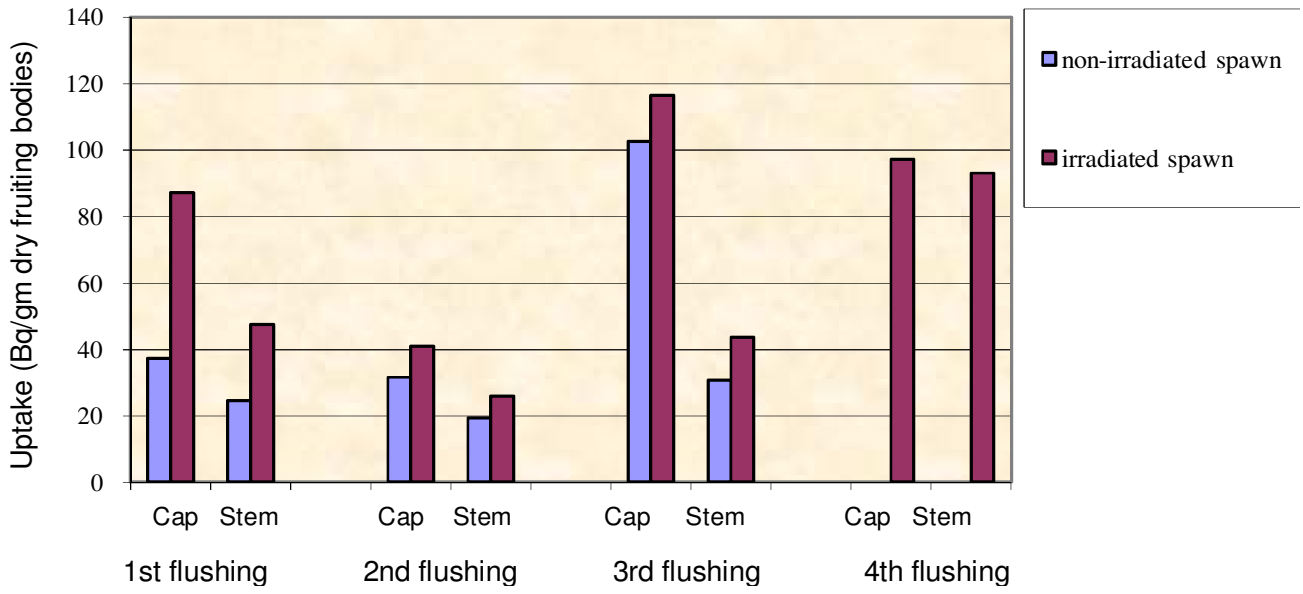


Figure 2. Effect of irradiation treatment of the *P. pulmonarius* spawn on the bioaccumulation of radiocesium-137 from spiked solid cellulose mixture waste substrate.

Table 6. Weight reduction percentage and degradation rats (g/day) for solid cellulose- based waste simulate by *P. pulmonarius*.

CBW	Weight reduction (%)	Degradation rats g/day
Unspiked	42.9	0.72
Spiked with Cs-137	58.0	0.97
Spiked with Co-60	54.5	0.91
Spiked with both Cs-137 and Co-60	58.6	0.97

should be mentioned that, for the irradiation treatment of spawn before inoculation, the bioaccumulation of radiocesium-137 is also higher in the caps compare to that in stem Figure 2. The same result were reached for the non- irradiated spawn (Table 4).

More than 15% weight reduction percentage was achieved in biodegrading the spike CBW compared to that of unspike one (Table 6). On the other hand, weight reduction percentages of the solid radioactive CBW degraded by *P. pulmonarius* is slightly varied based on the types of radiocontaminats. The degradation rate (g/day) for the solid cellulose- based waste exhibited similar trend (Table 6).

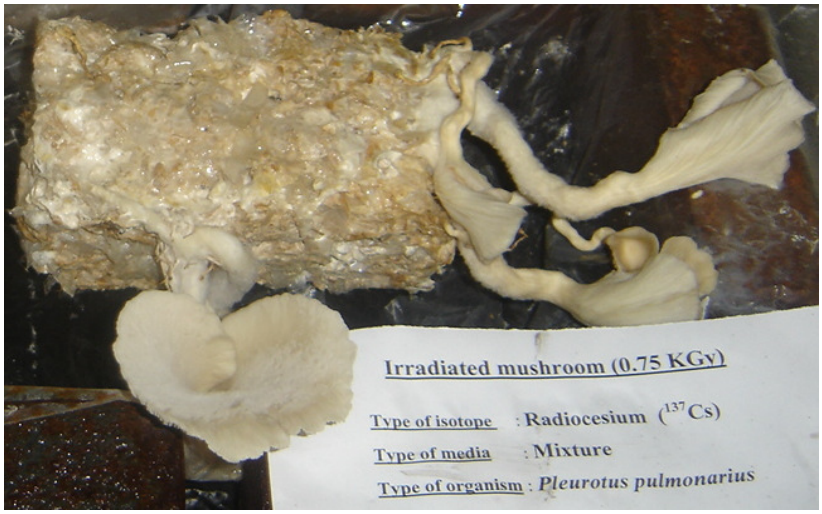
It should be noted that the weight reduction and degradation rate values for spiked substrates were highly comparable to that of non-spiked ones, which may refer to the internal irradiation doses received by the micro-organisms due to the two gamma emitters (Cs-137 and Co-60) found in the substrate (Figure 3a to c). These received doses enhance the capability of mushroom for the degradation of CBS. Comparable tendency was obtained in our previous published works (El-sayyad, 2008).

Conclusion

Studies on the bioremediation of some radioactive cellulose based waste simulates, in experimental *in vivo* conditions, had resulted in development of two treatment operations:

1. Biodegradation of the solid organic moieties in the waste, through their consumption as a source of carbon and energy by fungi and consequently acceptable weight and volume reduction figures was reached.
2. Simultaneous, biostabilization of radiocontaminants found in the waste, within the organism cells.

Irradiation of the *P. pulmonarius* spawn before their cultivation enhances the bioaccumulation of Cs-137 and up to four flushing were collected. The work provides an introduction for cost effective, natural hope and hype environmentally friendly clean up technology based on



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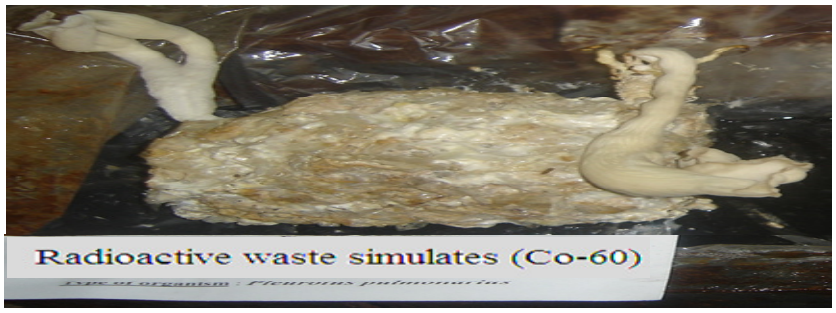

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Figure 3. *P. pulmonarius* cultivated on mixture of wastes contaminated with radiocesium and radiocobalt. (a) Mixture of waste contaminated with Cs-137, (b) Mixture of waste contaminated with Co-60, (c) Non-contaminated mixture of waste (control).

the capability of *P. pulmonarius* to process mixture of some categories of solid organic cellulose based hazardous wastes.

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