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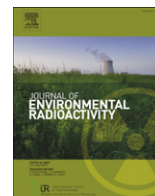
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Artificial and natural radioactivity in edible mushrooms from Sao Paulo, Brazil

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ABSTRACT

Environmental biomonitoring has demonstrated that organisms such as crustaceans, fish and mushrooms are useful to evaluate and monitor both ecosystem contamination and quality. Particularly, some mushroom species have a high capacity to retain radionuclides and some toxic elements from the soil and the air. The potential of mushrooms to accumulate radionuclides in their fruit-bodies has been well documented. However, there are no studies that determine natural and artificial radionuclide composition in edible mushrooms, in Brazil. Artificial (^{137}Cs) and natural radioactivity (^{40}K , ^{226}Ra , ^{228}Ra) were determined in 17 mushroom samples from 3 commercialized edible mushroom species. The edible mushrooms collected were *Agaricus* sp., *Pleurotus* sp. and *Lentinula* sp. species. The activity measurements were carried out by gamma spectrometry. The levels of ^{137}Cs varied from 1.45 ± 0.04 to $10.6 \pm 0.3 \text{ Bq kg}^{-1}$, ^{40}K levels varied from 461 ± 2 to $1535 \pm 10 \text{ Bq kg}^{-1}$, ^{226}Ra levels varied from 14 ± 3 to $66 \pm 12 \text{ Bq kg}^{-1}$ and ^{228}Ra levels varied from 6.2 ± 0.2 to $54.2 \pm 1.7 \text{ Bq kg}^{-1}$. ^{137}Cs levels in Brazilian mushrooms are in accordance with the radioactive fallout in the Southern Hemisphere. The artificial and natural activities determined in this study were found to be below the maximum permissible levels as established by national legislation. Thus, these mushroom species can be normally consumed by the population without any apparent risks to human health.

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1. Introduction

Artificial and natural radionuclides are found in diverse environmental compartments such as oceans, rivers, soils, rocks, vegetables and animal as well as human body tissues (Hu et al., 2010). Therefore, human beings and their environment are continuously exposed to these types of radiation, of which 81% can be attributed to natural radiation. The other 19% comes from artificial sources (Mazzilli et al., 2002).

Food is one of the main sources for elements and radionuclides for humans. This being true, radioactivity measurements in the environment and foodstuffs have become very important to evaluate the radiation levels to which man is exposed to either directly or indirectly.

Environmental biomonitoring has demonstrated that organisms such as crustaceans, fish and mushrooms are useful to evaluate and monitor both ecosystem contamination and quality (Marzano et al., 2001). Of these organisms, mushrooms stand out as they are excellent nutritional sources of proteins, fibers, vitamins and minerals, such as K, P and Fe and present low Na concentrations.

Presently, there are over 2000 known species, many of which are edible. However, some species are highly toxic and poisonous and if consumed can lead to death (Keizer, 2001).

According to Kuwahara et al. (2005) and Bazala et al. (2008), different mushroom species have the capacity to retain high concentrations of radionuclides and metals from the soil. These authors state that mushroom's longevity and wide mycelium make the mushroom an excellent environmental bioindicator. Among those radionuclides that can be accumulated by mushrooms are ^{40}K , ^{137}Cs , ^{226}Ra and ^{228}Ra .

Thus, the bioaccumulation characteristics of mushrooms can be used to detect even very low contamination levels. After the Chernobyl event, mushrooms have been used by many researchers as important biomonitors for high artificial radionuclide contamination. Battiston et al. (1989) analyzed the *Clitocybe infundibuliformis*, *Cantharellus lutescens* e *Boletus cavipes* edible mushroom species and found ^{137}Cs concentrations from 95 to 27,626 Bq kg^{-1} . According to these researchers, these high ^{137}Cs levels were not only related to soil contamination, but also due to the fact that some mushroom species presented high affinity to concentrate some radionuclides.

Kammerer et al. (1994) determined ^{137}Cs level in 83 wild mushroom species in three different regions of Germany. The levels varied from 2 to 15,000 Bq kg^{-1} and the *Xerocomus badius* species presented the highest ^{137}Cs level.

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Gasó et al. (2000) determined ^{137}Cs and ^{40}K medium activity values as 5.5 Bq kg^{-1} and 101.6 Bq kg^{-1} , respectively in 30 edible mushroom species collected in 4 Mexican cities. The maximum values obtained for ^{137}Cs and ^{40}K in these samples were below those reported values for some European countries.

Nowadays, mushrooms are being used in many studies related to radioecology. Furthermore, mushroom consumption in many countries is growing due to refined flavor, nutritional and medicinal values. From 1995 to 2005 the world's mushroom production increased more than 60%, which justify more studies in this area.

This growing world consumption of mushrooms can be attributed to intensive marketing of their nutritional and health benefits. This could have an effect on eating habits and reduce the cost of mushroom production. Studies have demonstrated that these organisms can reduce cholesterol levels, tumors, viral activity and also reduce the effects of thrombosis (Baeza et al., 2004).

On the other hand, studies have revealed high toxic element concentrations and high radionuclide levels in various mushroom species, especially in European countries. This fact led to the necessity of establishing the permissible maximum levels of radionuclides and toxic elements in this foodstuff. However, little attention has been given in determining the radioactive content in mushrooms and the respective ingestion dosage (Melquiades and Appoloni, 2004).

In the Southern Hemisphere, specifically in Latin American countries, there are only few studies on the evaluation of natural and artificial radioactivity in mushrooms. In Brazil, for example, there are no studies concerning as to the quality of cultivated Brazilian mushrooms. The ingestion of mushrooms, in Brazil, is still low compared to the European countries, where mushroom consumption is high and where families include mushroom cultivation as a domestic activity.

In this study, artificial (^{137}Cs) and natural radioactivity (^{40}K , ^{226}Ra , ^{228}Ra) were determined in 17 mushroom samples from 3 commercialized edible mushroom species in São Paulo, Brazil.

2. Materials and methods

2.1. Sampling

The edible mushroom samples were acquired in different commercial points of the São Paulo metropolitan region, specifically in Municipal Markets. Some samples were acquired directly from producers located in some cities as Mogi das Cruzes, Mirandópolis, Suzano and Juquitiba.

About 400 g were collected for each edible species *in natura*. The edible mushrooms collected were from *Agaricus bisporus*, *Agaricus blazei*, *Agaricus sp*, *Lentinula edodes*, *Pleurotus ostreatus*, *Pleurotus eryngii* e *Pleurotus ostreatoroseus* species. The whole mushroom was analyzed and no distinction was made between the parts of the mushrooms.

2.2. Sample preparation

All samples were cleaned and left submerged for 10 min in Milli-Q H_2O with $18 \text{ M}\Omega \text{ cm}^{-1}$ resistivity (Millipore®, Milford, USA). After this, the water was thrown out and the mushroom samples were cut in small pieces with a plastic knife and put in Petri plates or plastic recipients. The samples were then freeze-dried for 10–15 h at $-51 \text{ }^\circ\text{C}$ in a ModulyD Model freeze-dryer (Thermo Electron Corporation, Milford, USA). After freeze drying, the samples were ground and homogenized in a domestic blender with Ti blades.

2.3. Gamma spectrometry

About 20 g of each freeze-dried mushroom sample were placed in a polyethylene bottles previously cleaned with 10% nitric acid (Merck, Darmstadt, Germany) and Milli-Q water. The bottles (65 mm of diameter and 19 mm of height) were stored for approximately 35 days until the respective counting. The activities of gamma emitters were determined by using a High purity Ge (HPGe) POP TOP Model detector (EG&G ORTEC, Oak Ridge, USA) with 1.9 keV resolution for the 1332.2 keV of ^{60}Co and 50% relative efficiency.

2.4. ^{137}Cs , ^{40}K , ^{228}Ra and ^{226}Ra determination

To determine the low ^{137}Cs levels the methodology developed by Figueira et al. (2007) was applied. Consequently, the same methodology was used for ^{40}K , ^{226}Ra and ^{228}Ra determinations.

^{137}Cs (661.6 keV) and ^{40}K (1460.8 keV) were determined directly by their own photopeaks. In the case of ^{226}Ra and ^{228}Ra , both were determined through their daughter radionuclides: ^{214}Bi (609.3 keV) and ^{228}Ac (911.2 keV), respectively.

The adopted procedure consisted, initially, in an accumulating counting of background radiation (BG) measuring one empty plastic bottle in intervals of 25,000 s, from 100,000 to 200,000 s. Later, reference materials that were placed in the same geometric bottles and stored up to 20 days, were submitted to the same counting procedure for the BG, in order to calculate the detector efficiency.

Linear regression fits were made for the activity by counting time (BG and reference materials). Then, the detector efficiency was determined by Eq. (1):

$$\varepsilon = \frac{a_{\text{RM}} a_{\text{BG}}}{m_{\text{RM}} \times A_{\text{RM}}} \quad (1)$$

where ε is the efficiency for the radionuclide determined a_{RM} ; a_{BG} is the angular coefficient of the regression curve for the reference material and BG; m_{RM} is the reference material mass, in kilograms; A_{RM} is the reference material activity concentration (Bq kg^{-1}), adjusted to the date of the analysis.

The same accumulative method used for the reference materials was used for the sample counting. The activity concentrations were determined from Eq. (2):

$$A = \frac{a_{\text{S}} a_{\text{BG}}}{m_{\text{A}} \times t \times \varepsilon} \quad (2)$$

A is the sample activity concentration in Bq kg^{-1} ; a_{S} , a_{BG} is the angular coefficient of the regression curve for the sample and BG; m_{A} is the sample mass in kg; t is the counting time in seconds; ε is the efficiency for the radionuclide determined.

3. Results and discussion

The detector efficiency (ε) was obtained from measurements of reference materials: IAEA-300 (sediment marine), for ^{137}Cs and ^{40}K and IAEA-300 (sediment marine) and IAEA-327 (soil) for ^{226}Ra and ^{228}Ra . The reference material data used for the efficiency determination and the efficiency values obtained are presented in Tables 1 and 2.

For the validation of the methodology IAEA-307 (sea plant), IAEA-414 (mixed fish), IAEA-375 (soil) and IAEA – Mushroom reference materials were analyzed. The mushroom reference material was prepared within the frame of an IAEA Interregional Technical Cooperation Project (Waheed et al., 2007; Polkwska-Motrenko and Rossbach, 2007). The results are presented in

Table 1
IAEA reference material data for ϵ values of ^{137}Cs and ^{40}K .

Reference material	Reference date for decay correction	Certificated value (Bq kg ⁻¹)		Efficiency values (%)	
		^{137}Cs	^{40}K	^{137}Cs	^{40}K
Marine sediment IAEA –300	01/01/1993	1066.6 (1046–1080)	1059 (1046–1226)	1.90	0.19

Table 2
IAEA reference material data for ϵ values of ^{228}Ra and ^{226}Ra .

Reference material	Reference date for decay correction	Certificated value (Bq kg ⁻¹)		Efficiency values (ϵ)	
		^{228}Ra	^{226}Ra	^{228}Ra	^{226}Ra
Marine sediment IAEA 300	01/01/1993	61.6 (59.0–63.9)	56.5 (54.4–60.2)	0.73	1.19
Soil IAEA 327	31/12/1994	38.7 (37.8–39.6)	34.1 (32.7–35.5)	0.76	1.56

Table 3. The relative standard deviation values varied from 0.41 % to 18.5% and the relative error values varied from 0.7 to 15.6%.

The minimum detection concentration was calculated by Eq. (3) (IAEA, 1989) for each radionuclide:

$$\text{CMD} = \frac{4.66 \times S_B}{t \times \epsilon \times m} \quad (3)$$

where S_B is the standard deviation of counting rate for the blank, in Bq kg⁻¹; t is the counting time, in seconds; ϵ is the detector efficiency; m is the sample mass, in kg.

The minimum detection activity concentrations (MDC) obtained were: 25.6 Bq kg⁻¹ for ^{40}K , 1.1 Bq kg⁻¹ for ^{137}Cs , 7.3 Bq kg⁻¹ for ^{226}Ra and 3.1 Bq kg⁻¹ for ^{228}Ra .

Seventeen edible mushroom samples were analyzed and the activities (Bq kg⁻¹ in dry weight) for ^{40}K , ^{137}Cs , ^{228}Ra and ^{226}Ra are presented in Table 4.

The activity concentration of ^{137}Cs varied from 1.45 ± 0.04 to 10.6 ± 0.3 Bq kg⁻¹ (dry weight). According to Gillet and Crout (2000), different factors determine the ^{137}Cs levels in mushroom as well as the ability of some species to retain this radionuclide such as depth of mycelium and substrate cultivation.

The ^{137}Cs levels determined in the edible mushroom species were lower than the reported levels by Teherani (1988) in Austria that analyzed several European mushroom species a year after the Chernobyl accident. The levels determined varied from 18 to 3852 Bq kg⁻¹.

Gasó et al. (2000) analyzed wild mushroom in Mexico and the specific activities found varied from 0.2 to 91 Bq kg⁻¹. In Poland, Malinowska et al. (2006) studied edible mushroom and the levels found were 330–6670 Bq kg⁻¹. This variation of activity can be related to the fallout difference between the Northern and Southern Hemispheres.

However, according to Martine and Ramsey (2008) to evaluate the ^{137}Cs accumulation in mushrooms it is important to consider the species, since several species have high capacity to retain this radionuclide.

Table 3
 ^{40}K , ^{137}Cs and ^{226}Ra activities (Bq kg⁻¹) in the reference materials.

Reference material	^{40}K		^{137}Cs		^{226}Ra	
	This study	Certified value	This study	Certified value	This study	Certified value
IAEA Mushroom	962 ± 4 (15.3) ^a	1136 (1046–1226) ^b	3184 ± 94 (9.8) ^a	2899 (2740–3058) ^b	–	–
IAEA-414	484 ± 2 (0.7) ^a	480 (461–498) ^b	–	–	–	–
IAEA-307	–	–	5.5 ± 0.2 (12.5) ^a	4.9 (4.5–5.2) ^b	–	–
IAEA-375	–	–	–	–	23.7 ± 4.4 (15.6) ^a	20 (18–22) ^b

The reference materials used do not possess certified values for ^{228}Ra .

^a RE = Relative error.

^b 95% Confidence interval.

The maximum value for ^{137}Cs obtained was 10.6 ± 0.3 Bq kg⁻¹ which was lower than the allowed level by Brazilian legislation (CNEN-301/006), which is 1000 Bq kg⁻¹ for foodstuffs.

In regards to ^{40}K in Brazilian edible mushrooms, all species presented the activity concentrations for this radionuclide and the levels varied from 461 ± 2 to 1535 ± 10 Bq kg⁻¹ (dry weight).

On the other hand, unlike ^{137}Cs , ^{40}K activity levels were comparable with other studies. Wang et al. (1998), in Taiwan, found levels ranging from <50 to 1230 Bq kg⁻¹ and Malinowska et al. (2006) in Poland reported values between 180 and 1520 Bq kg⁻¹ (dry weight). These studies seem to suggest that ^{40}K incorporation is self-regulated by fungi, whereas ^{137}Cs appears not to be regulated by fungi. Kuwahara et al. (2005) proposed that the ^{40}K variations in mushroom are related to local geology, cultivation methods, use of fertilization, culture age, among other factors.

The ^{226}Ra activity concentrations in the edible mushroom species analyzed varied from 14 ± 3 to 66 ± 12 Bq kg⁻¹ and the activity concentrations of ^{228}Ra varied from 6.2 ± 0.2 to 54.2 ± 1.7 Bq kg⁻¹ (dry weight). To the best of our knowledge there are very few published studies reporting the activity concentration of ^{226}Ra and none found concerning ^{228}Ra levels in mushrooms.

The ^{226}Ra activity concentrations found were higher than that reported by Baeza et al. (2005) in Spain whose values varied from below the detection limit (in most samples) to 62 ± 35 Bq kg⁻¹ (dry weight). For comparison purposes the concentrations of Ra-isotopes in vegetables in general found in literature varied from 0.6 ± 0.3 to 17 ± 7 Bq kg⁻¹ for ^{226}Ra and 0.3 ± 0.04 to 17 ± 5 Bq kg⁻¹ for ^{228}Ra according to Shanthi et al. (2010) in Indian vegetables, fruits and tuber. Pietrzak-Flis et al. (2001) found lower and higher ^{226}Ra activity concentrations from 8.66 ± 0.29 to 137 ± 2 Bq kg⁻¹ in cucumber and parsley, respectively, among a group of vegetables. Lauria et al. (2009) measured black beans, lettuce and carrot activity concentrations in the range of 28–93 Bq kg⁻¹ for ^{226}Ra and 36–117 Bq kg⁻¹ for ^{228}Ra .

All the activity concentrations measured for Ra-isotopes in Brazilian mushrooms lie within the range of vegetable foodstuffs as

Table 4
Activities for de ^{40}K , ^{137}Cs , ^{226}Ra and ^{228}Ra in edible mushroom samples (dry weight).

Sample code	Origem	Specie	^{40}K (Bq kg $^{-1}$)	^{137}Cs (Bq kg $^{-1}$)	^{226}Ra (Bq kg $^{-1}$)	^{228}Ra (Bq kg $^{-1}$)
CCG	São Paulo-SP	<i>Agaricus bisporus</i>	764 ± 3	1.8 ± 0.1	<7.3	27.0 ± 0.9
CML	Mogi das Cruzes-SP	<i>Agaricus bisporus</i>	743 ± 3	<1.1	<7.3	6.2 ± 0.2
CCM	Mogi das Cruzes-SP	<i>Agaricus bisporus</i>	752 ± 3	<1.1	<7.3	<3.1
CAD	São Paulo-SP	<i>Agaricus blazei</i>	860 ± 4	1.45 ± 0.04	<7.3	<3.1
PBL	São Paulo-SP	<i>Agaricus sp.</i>	1215 ± 5	8.6 ± 0.3	18 ± 3	38.1 ± 1.2
SPL	São Paulo-SP	<i>Lentinula edodes</i>	813 ± 3	1.7 ± 0.1	14 ± 3	14.1 ± 0.5
SJL	Juquitiba – SP	<i>Lentinula edodes</i>	806 ± 3	6.0 ± 0.2	<7.3	11.3 ± 0.4
SMM	Mirandópolis-SP	<i>Lentinula edodes</i>	461 ± 2	3.5 ± 0.1	<7.3	<3.1
SML	Mogi das Cruzes-SP	<i>Lentinula edodes</i>	887 ± 4	2.6 ± 0.1	23 ± 4	35.6 ± 1.1
CSD	São Paulo-SP	<i>Lentinula edodes</i>	713 ± 3	4.9 ± 0.1	<7.3	16.4 ± 0.5
CJD	São Paulo-SP	<i>Lentinula edodes</i>	841 ± 4	3.3 ± 0.1	<7.3	<3.1
CSE	São Paulo-SP	<i>Pleurotus ostreatus</i>	849 ± 4	6.7 ± 0.2	24 ± 4	26.9 ± 0.9
CHH	São Paulo-SP	<i>Pleurotus ostreatus</i>	703 ± 3	3.6 ± 0.1	15 ± 3	29.0 ± 0.9
CES	São Paulo-SP	<i>Pleurotus eryngii</i>	797 ± 3	<1.1	17 ± 3	12.6 ± 0.4
CEU	Suzano-SP	<i>Pleurotus eryngii</i>	785 ± 3	3.8 ± 0.1	23 ± 4	14.9 ± 0.5
CSI	São Paulo-SP	<i>Pleurotus ostreatoroseus</i>	1535 ± 10	10.6 ± 0.3	66 ± 12	54.2 ± 1.7
SFZ	São Paulo-SP	<i>Pleurotus ostreatoroseus</i>	566 ± 2	3.5 ± 0.1	18 ± 3	9.7 ± 0.3

reported in literature. According to UNSCEAR (2000) reference values for ^{226}Ra and ^{228}Ra are 0.05 and 0.04 Bq kg $^{-1}$ for leafy vegetables and 0.03 and 0.02 Bq kg $^{-1}$ for root vegetables and fruits. The activity concentrations vary in a range from 0.002 to 1.150 Bq kg $^{-1}$ for ^{226}Ra and from 0.11 to 0.22 Bq kg $^{-1}$ for ^{228}Ra in the same products.

There are many factors affecting the level of radioactivity in mushrooms. Some of these include soil, pH and species of mushrooms according to Yoshida et al. (1994). Furthermore, atmospheric conditions, such as rainfall can also exert influence up on radioactivity levels.

As stated above, since some studies suggest that the presence of natural and artificial radionuclides in edible mushroom samples is related to the type species of these organisms, as well as farming and environmental influences. Based on this, the normalized results were evaluated by cluster analysis with samples organized based on their degree of similarity, in order to classify them into groups (Fig. 1).

From the cluster analysis three groups were identified: G1, G2 and G3. In general, analyzing the composition of these groups, all three mushroom species was found in each group. The groups were then separated according to the activity concentrations. The G3 samples had the highest concentrations of radionuclides in relation to other groups.

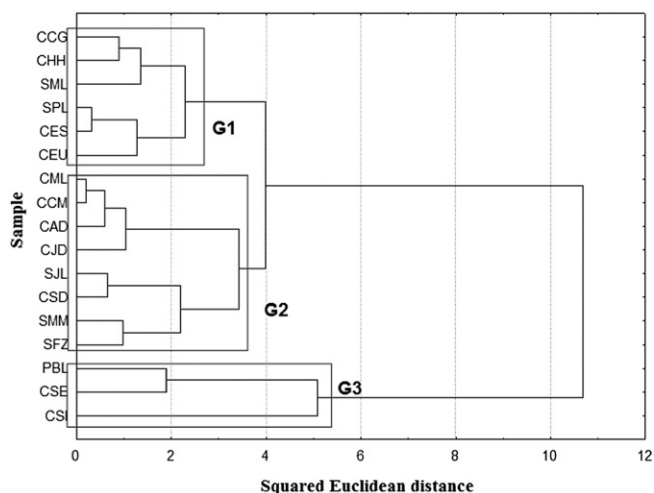


Fig. 1. Cluster analysis of the edible mushroom samples.

It was observed that for high values of ^{137}Cs and ^{40}K both presented the same behavior, e.g. same chemical behavior. The limited number of samples did not allow the verification of the origin source of radionuclides. But it is known that agricultural processes and fertilizers can significantly influence the content of radionuclides. This same heterogeneity is also observed in relation to the sample origin. In this case the variation obtained can be attributed directly to the form of cultivation, relating to the use of certain fertilizers that can significantly influence the radioactive contents. As it is known, fertilizers can contain different radionuclides in their composition (Luca et al., 2009; Bituh et al., 2009).

4. Conclusion

The gamma spectrometry methodology allowed to determining the ^{137}Cs , ^{40}K , ^{226}Ra and ^{228}Ra levels present in edible mushroom samples with good precision and accuracy. The ^{137}Cs levels in the edible mushrooms are in accordance with the Southern Hemisphere fallout level. The highest value found in this study for the activity of ^{137}Cs was very low in comparison with the maximum allowed by national legislation for foodstuffs. ^{40}K levels obtained are in agreement with values given in other studies. Thus, the edible mushrooms analyzed in this study do not present a risk to their consumers, even though consumption of this type of foodstuff is still small in Brazil. However, it is always necessary to establish controls not only the concentration of ^{137}Cs in mushrooms, but also in other foods such as meat, milk, fruit, etc. In the case of ^{40}K , which is evenly distributed in the body and is under homeostatic control, is less dangerous to human health than ^{137}Cs . The ^{228}Ra and ^{226}Ra levels obtained were also comparable with literature values.

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