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Influence of alkali and alkaline earth elements on the uptake of radionuclides by *Pleurotus eryngii* fruit bodies

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ABSTRACT

In the literature, there are many data available on radionuclide contents and their transfer to different species of mushrooms. There are some variables, however, which affect the transfer but are very difficult to observe in collected wild mushrooms. An example is the effect of different concentrations of alkali and alkaline earth elements in the soil. Modification of these concentrations in the soil solution has traditionally been used as a countermeasure to deal with radioactively contaminated areas. In the present work, fruiting bodies of *Pleurotus eryngii*, a saprophytic mushroom, were grown under controlled laboratory conditions, varying the content of alkali (potassium and cæsium) and alkaline earth (calcium and strontium) elements. The transfer of ^{134}Cs, ^{85}Sr, and ^{60}Co (added to the cultures) and of natural ^{210}Pb was analysed by increasing the content of each stable element considered. A significant, but nonlinear, enhancement of stable cæsium and ^{134}Cs was observed with increasing content of stable cæsium in the substrate/mycelium. The transfer of ^{85}Sr decreased with the addition of each stable cation, whereas the ^{60}Co and ^{210}Pb transfers were unaffected.

1. Introduction

Mushrooms are known to accumulate radionuclides, although there is wide variability—up to seven orders of magnitude in the case of radiocæsium (Baeza and Guillén, 2007). Knowledge of the uptake of radionuclides by fungi is usually based on studies of their content in wild mushrooms collected in natural or semi-natural environments. The most studied radionuclides were the radiocaesium isotopes (Gillett and Crout, 2000; Baeza and Guillén, 2007; Duff and Ramsey, 2008). Naturally occurring radionuclides are also present in the fruit bodies, but the study of their content had been somewhat overlooked in favour of anthropogenic radionuclides. The content of ^{210}Pb and its descent ^{210}Po, which had great radiotoxicity, were responsible for the greatest contribution. In environments not heavily contaminated by radionuclide fallout, it was even higher than that of radiocaesium (Guillén et al., 2009; Vaarama et al., 2009).

On the other hand, some radionuclides that currently are practically absent in the environment but may at some time in the future be released in the case of a hypothetical nuclear accident, cannot be assessed, nor their transfer, by the analyses of wild mushrooms. Another example of this limitation is the effect that stable cations added to the soil may have on the transfer of radionuclides. Sometimes it is difficult to determine it using only information on the radionuclide content of wild mushrooms. Indeed, such additions have frequently been applied to radioactively contaminated soils as countermeasures aimed at reducing the transfer of the contaminants to plants. In particular, inorganic fertilizers have been used based on the idea of saturating the soil solution with stable cations that are chemical analogues of the radionuclides involved, i.e., fertilizers that release potassium in the case of radiocæsium contamination, and calcium in the case of radiostrontium (Nisbet et al., 1993; Fesenko et al., 2007). In some areas of Sweden, a single addition of K-based fertilizer (100 kg K/ha) was used to prevent radiocaesium uptake. Almost two decades years later, it was observed an apparent decrease of radiocaesium accumulation by mushrooms in treated areas, although less pronounced than in vascular plants and dependant of the species of mushroom (Rosén et al., 2011).

In these cases, the use of cultures under controlled laboratory conditions could be of use, although such cultures are complicated to perform and are not possible for all species of fungi. In the present case, we selected the species *Pleurotus eryngii* because it is representative of saprophytic fungi and there are culture techniques available which yield fruit bodies (Ferri, 1985; Baeza et al., 2000). The aim of the present study is to analyse the dependence of the uptake of the man-made radionuclides (^{134}Cs, ^{85}Sr, and ^{60}Co) and of the naturally occurring radionuclide ^{210}Pb by fruiting bodies of the saprophytic fungus *Pleurotus eryngii* with varying concentrations of alkali (K and Cs) and

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alkaline earth (Ca and Sr) elements in the substrate/mycelium in which they grow. These radionuclides were selected firstly because two of them are chemically identical to ^{137}Cs and ^{90}Sr; secondly because ^{60}Co is not usually present in the environment, but may be released in a hypothetical accident; and thirdly because ^{210}Pb is a uranium series radionuclide that is a predecessor of ^{210}Po, and can have a great radiological significance in areas not heavily contaminated with anthropogenic radionuclides (Guillén et al., 2009; Vaarama et al., 2009).

2. Material and methods

2.1. Mushroom culture under controlled laboratory conditions

Mushrooms of the edible species *P. eryngii* were grown under controlled laboratory conditions, following a procedure explained in detail elsewhere (Baeza et al., 2000). It basically consisted of two consecutive phases:

a) The spread of the mycelium in the substrate from which it obtained its nourishment. The fungal material needed to inoculate the substrate consisted of sterilised wheat grains which were inoculated with *Pleurotus eryngii* strain and cultivated for 20 days at 25 °C. The substrate consisted of 60 g d.w. of wheat grain, 30 g f.w. straw, 10 g d.w. of wheat flour, and 20 g d.w. of calcareous soil of pH=7.2. The addition of the corresponding treatments (K, Ca, Cs, and, Sr) were carried out during the preparation of the substrate, prior to its inoculation with fungal material. The subtrate was inoculated with the prepared fungal material, and let it to expand for 20–30 days in darkness at 25 °C.
b) Transferring the substrate with the mycelium to a soil bed for the subsequent formation of the fruiting bodies. The expanded mycelium from the previous phase was placed on a 40 mm depth bed of soil in square trays. Then it was covered with another layer of soil, 15 mm depth. The trays were placed in a cultivation chamber with cold lighting, in which the three variables temperature, humidity, and photoperiod were controlled. These are fundamental parameters for the growth of the mushroom in reproducible conditions. The substrate was watered to saturation morning and evening by means of a fine spray, using roughly 80 mL water per irrigation. The resulting average humidity was 75%. The temperature was maintained at 14.0 °C during the hours of darkness, and 18.0 °C during light hours, the photo-period being fixed at 16 h.

The fruiting bodies were harvested by cutting the stipe slightly above soil level so as to have no soil particles adhered to the samples to be analysed. Known activities of ^{134}Cs, ^{85}Sr, and ^{60}Co (about 450, 33 000, and 2000 Bq, respectively) were incorporated onto the surface of the substrate/mycelium when the mycelium was in the spreading phase 10 days after its inoculation into the substrate (see Fig. 1). The mass equivalents of the radionuclides added were 9.41×10^{-12}, 3.76×10^{-11}, and 9.40×10^{-11} g for ^{134}Cs, ^{85}Sr, and ^{60}Co, respectively. Similar activities have been added in other laboratory studies (Dighton et al., 1991). All the radionuclides were added as chlorides in previously sterilised solutions, and were conditioned to neutral pH at the time of their addition to the culture medium so as not to damage the mycelium's growth (Baeza et al., 2000). The method of incorporation was by uniformly distributing many small droplets over the substrate/mycelium. This incorporation route was designed to simulate the radioactive contamination of an ecosystem, in which man-made radionuclides are normally deposited onto the surface layer of the soil by rainfall, and then migrate down to the depth at which the mycelium is located (Baeza et al., 2000). For the analysis of ^{210}Pb, no additional radionuclide was added, with its content in the substrate/mycelium (0.208 ± 0.025) Bq/Experience being the only source of this radionuclide.

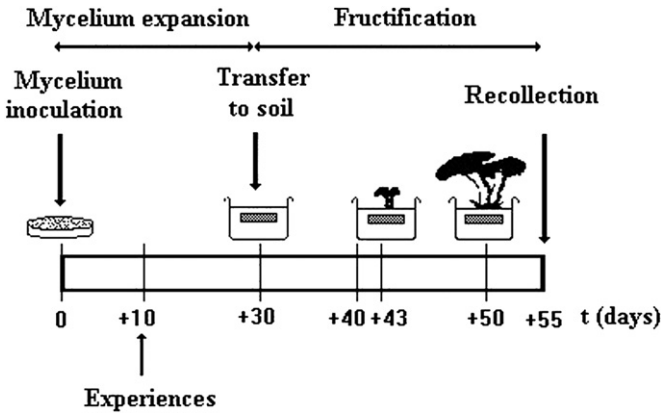

Fig. 1. Chronogram of the culture of *P. eryngii* under laboratory conditions corresponding to Experiments A, K1, K2, Ca1, Ca2, Cs1, Cs2, Sr1, and Sr2. Time zero corresponds to the inoculation of the mycelium into the substrate.

Nine sets of experiences were designed and carried out in triplicate: (i) Experience A in which mushrooms were grown without the addition of any supplementary K, Ca, Cs, or Sr; (ii) Experiences K1 and K2, in which 2.36 and 4.78 mg/g d.w. of K_2CO_3 were added to the substrate; (iii) Experiences Ca1 and Ca2, in which 6.3 and 12.7 mg/g d.w. of $CaCO_3$ were added to the substrate; (iv) Experiences Cs1 and Cs2, in which 6.6 and 13.2 μg/g d.w. of CsCl were added to the substrate; and (v) Experiences Sr1 and Sr2, in which 0.55 and 1.14 mg/g d.w. of $Sr(NO_3)_2$ were added to the substrate. The added quantities of K, Ca, Cs, and Sr were calculated to provide an increase of the concentrations of these elements in the soil similar to those observed in soils by Yoshida and Muramatsu (1998).

2.2. Radionuclide determination

The mushroom samples were dried in a flow of hot air at 90 °C, homogenised, and put into Petri dishes of 52 mm diameter and 13 mm depth for the gamma spectrometry. This was performed using an n-type Ge intrinsic detector with 25.6% efficiency, 1.85 keV resolution, and a peak-to-Compton ratio of 57:1, all referred to the 1.33 MeV ^{60}Co gamma emission. This detector was coupled to a Compton effect suppressing device which enables the background (due largely to the Compton scatter of photons from ^{40}K present in the samples) to be reduced by a factor of 2 in the 661.6 keV energy region of the ^{137}Cs emission. The Compton suppressing device was capable of simultaneously acquiring two different spectra: (i) suppressed, in which the suppressor was turned on for the determination of simple peak gamma emitters such as ^{85}Sr, and (ii) normal, in which the suppressor was turned off for the determination of multiple gamma emitters, such as ^{134}Cs and ^{60}Co (López et al., 1998). The detection limit was about 0.030 Bq/kg for a period of 48 h.

For the determination of ^{210}Po in solid samples, aliquots of 1 g dry weight were digested with HNO_3 and H_2O_2 (8:2 mL) in a microwave oven (Ethos Pro Milestone Ltd.) at 180 °C for 20 min. The sample was filtered, evaporated, and converted to HCl 1.5 M medium. ^{208}Po was used as tracer. The polonium from solid or water samples was finally autodeposited onto silver planchets, and measured by α-spectrometry (Bolívar et al., 2002). The detection limit was about 1 Bq/kg d.w. The average recovery was 35%. Alpha spectrometry was carried out using twelve silicon