

# A simple and quick model to study uptake and transfer of radionuclides and heavy metals from mycelium to the fruitbody of saprophytic edible fungi

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**Abstract** A simple model of *Pleurotus eryngii* mushroom culture, grown under stringent laboratory conditions, was developed to watch ecophysiological pathways of xenobiotics in saprophytic fungi. The investigated substances may be added in different stages of biological cycle of the fungus. It is emphasized that to obtain the fruitbodies, all the physiological needs of the species have to be fulfilled, i.e.: nutritional requirements, optimal temperature (according to the biological cycle), humidity, aeration (oxygen and CO<sub>2</sub>), absence or presence of the light in each reproduction phase, as well as the control of infections and plagues through all the production stages. The described model serves for investigation of radionuclide and heavy metal uptake and transfer in fungi. Double or some multiple fructification from the same substrate is possible giving a possibility to investigate bioremediation by mycoextraction.

**Key words** bioaccumulation • radionuclides • heavy metals • mushrooms • mycoextraction • *Pleurotus eryngii* • saprophytic fungi

## Consumption of mushrooms in the past

Large in dimensions fungi, which we call mushrooms, are much less recognized by the biotechnological, chemical and pharmaceutical industry than their tiny or microscopic relatives (e.g. moulds or yeasts). Although, talking about mycoetnology and eventually mycogastronomy, they have been known in Europe since 3325–3100 BC, what we know thanks to the finding of the “man of Hauslabjoch”, “man of Ötzi”, or “iceman” [3, 9], found in a glacier of the Alps, in the region of Tirol (Italian-Austrian frontier). The well-conserved prehistoric man was holding a wood-rot mushroom in his chaff, identified as *Piptoporus betulinus*. This fungus could be utilized as a tinder to set the fire or as a source of an antibiotic with a weak antibacterial action. Till the revelation of the finding of the “iceman” some documental data on consumption of mushrooms in Europe was poisoning of a mother and her three sons in the play of Ikar (Euripides) from 450 BC, as well as the poisoning of the emperor Claudius in 54 BC. The Romans knew and gave the names to many fungi, as for example the wood-rot fungus *Fomes officinalis* discovered for its medicinal properties or “delicatessen” “Boleti” (*Amanita caesarea*), “Suilli” (*Boletus edulis*) or truffles. And all the wild mushrooms that were consumed traditionally till 1650 and 1700, when the technique of the culture of champignon came to life in Paris.

Out of Europe, in the Far East, various fungi were ingested during the centuries, *Lentinula edodes* “shiitake” and *Auricularia polytricha*, or by American Indians – *Cyttaria* spp. Therefore, since the most remote antiquity man has used fungi as a part of his diet and because of

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Received: 26 August 2004

their medicinal properties. Numerous mushrooms have also been described by Japanese and Chinese traditional medicine for more than 4000 yrs., as in case of the fungus “Ling Zhi” in China or “Reishi” in Japan (*Ganoderma lucidum*).

Nowadays, an increase of mushroom consumption is evoked by both gastronomic virtues of fungi and growing interest in the natural environment. These wild species that strongly depend on appropriate climatic conditions (humidity, temperature etc.), throughout the fructification processes, may present a great gastronomic value, be abundant or rare, and collected by amateurs or professionals who have commercialized them. Therefore, the collections of mushrooms and fungi in the countryside needs to be regulated adequately, since they can cause some environmental problems (environmental protection), sanitary problems (heavy metal accumulation) etc.

### Introduction to the cultures of saprophytic fungi

Saprophytic fungi are easier to cultivate than parasitic and mycorrhizal ones since they have less stringent nutritional requirements. They use organic compounds derived from microbial residues from dead plants or animals, or if alive, fungi can use their exudates spread in the environment.

The culture of fungi, because of its technological complexity has not followed the human development throughout the centuries. In principle, man was a collector only, then he dominated agriculture and livestock farming. However, even nowadays, only few mushrooms and fungi are cultivated for their gastronomic and medicinal values, if compared to many agricultural plant species. We should also have in mind other applications as production of antibiotics, forestry (mycorrhizal plants), and the fermentation processes (to produce alcohol) etc.

For that reason, the culture of fungi seems to be an alternative for the social demand of consumption which has been extended due to the technological progress that permits to obtain tasty mushrooms of the best quality out of the season and almost everywhere in the world.

To know how to cultivate a fungus, one has to possess necessary knowledge about its life cycle and trophic relations. Satisfactory understanding of its life also requires some profound knowledge of biology and microbiology. The fungal life cycle starts with the development of primary or secondary mycelium which is able to reproduce asexually and/or sexually, depending on the species and environmental conditions.

The appropriate preparation of a mixture of substrate, for its sterilization or composting, seems to be crucial step to get the desired mycelium development and its later fructification. For this reason, it is very important to select optimal substrates to make mycelium develop vigorously (“mycelium expansion” to form the “spawn”) and to obtain compact mass of mycelium.

It seems adequate to use easily accessible agricultural or industrial subproducts that are inexpensive, colonized very fast (an elevated biological efficiency) and equilibrated nutritionally, which contributes to the organoleptic conditions (flavour and taste), appropriate for the mushrooms. Moreover, one has to know the adequate relation inoculum/unit of substrate of expansion, which is sterilized or pas-



**Fig. 1.** *Pleurotus eryngii* degrading the rhizome of *Eryngium campestre*.

teurized; conditions of incubation, as well as control of the contamination of the substrate.

Thus, in the industrial level, the culture of macroscopic fungi and edible mushrooms consists of biotechnological control of saprophytic wild mushrooms (Fig. 1) with a long gastronomic tradition, as well as introduction of new selected stocks, e.g. hybrids, possessing optimal gastronomic and organoleptic features. Their origin may be domestic or international.

In general, to obtain the fructification of a fungus, all its physiological needs have to be fulfilled: nutritional requirements, optimal temperature (according to the biological cycle), humidity, aeration (oxygen and CO<sub>2</sub>), absence or presence of the light in each reproduction phase, as well as the control of infections and plagues through all the production stages.

### Fungi, radionuclides and heavy metals

The past few years brought an amount of alarming data on the contamination of edible mushrooms [2, 6, 7]. It is not only the elevated level of radionuclides (<sup>137</sup>Cs and <sup>134</sup>Cs), observed after the Chernobyl NPP accident but also enormous quantity of heavy metals detected in the environmental samples. Due to that, the World Health Organisation advised not to eat more than 250 g of fresh mushrooms per week [5]. Unfortunately, numerous collections take place in the highly polluted wayside and industrial areas including forests growing in the vicinity of national roads.

It is generally known that many fungal species accumulate metallic ions to an enormous extent, much more than plants do [7]. Concerning the determinations of the fate of common pollutants in the environment does not bring enough information on the mechanisms responsible for accumulation of them in mycelium. Thus in case of radionuclides compartmentation models were developed to describe their transfer [1] and connection with the transport of other elements, e.g. between cesium and potassium for which the Discrimination Factor was introduced [4].

There are very few communications on physiological approaches of elemental uptake and accumulation in fungi studied under controlled laboratory conditions [1, 10]. However, there is a growing need to perform numerous toxicological tests showing rates of xenobiotic accumulation

in the fruiting bodies of edible fungi. Also, application of mycelium blocks in bioremediation of contaminated water is possible. Tremendous accumulation of cesium in the fruiting mushrooms shows a possibility for mycoextraction of  $^{137}\text{Cs}$  from the contaminated soil. However, such a procedure needs to be optimized and all the biological and technical processes studied well. Last but not least, the molecular mechanisms responsible for that accumulation capacity have not been recognized yet. It is possible that, to some extent, they are homologous with those described in plants and animals.

This points to the necessity to develop culturing methods for the purpose of research in ecotoxicology and ecophysiology.

## Materials and methods

Heavy metals, radionuclides and other xenobiotics may be incorporated in different phases of the life cycle of *Pleurotus eryngii* (the model saprophytic fungus, Fig. 1), i.e. before the inoculation, after the inoculation, after the phase of mycelium expansion (in the interphase level), within primordial formation and in the maturation phase of basidiocarpus [1].

In agreement with preceding investigations [8], the following is a description of a simple protocol to obtain mycelium biomass (spawn) and fructification of *Pleurotus eryngii*, so as to obtain a liquid interface between the developed mycelium and the container where radionuclides can be added to observe its rapid transfer to the basidiocarpus via mycelium.

The applied model has undergone different phases while it was facilitated with all the steps of protocol and the materials used. It was not only due to make a simple model for common use but also to eliminate influence of many substrate constituents, minimize the amount of soil or peat and replace tap water with distilled water partially.

### Spawn preparation (*in vitro*)

1. Wash and move 10 kg of barley seeds with tap water properly.
2. Slip water from the washing.
3. Place and submerge clean barley in distilled water (eliminating floating seeds).
4. Leave for hydration for 24 h (and move from time to time).
5. Slip the barley from the excess of water well.
6. Distribute the hydrated barley among containers (for *in vitro* tissue culture, Duchefa Biochemie B.V., Haarlem, The Netherlands, OS 140 Box + ODS Filter, Cat. No. E 1670.0001 or similar) to the amount 450 g of hydrated barley per container which corresponds to 243 g of dry weight.
7. Autoclave the containers at 120°C during 2 h covered with aluminum paper.
8. Inoculate surface of the container next day with mycelium of *Pleurotus eryngii*, with approximately 15 g of inoculum (in sterile conditions) and cover with the cap. The strain was first isolated from a basidiocarp and grown on a Petri plate with a standard MA medium



Fig. 2. Mycelium development, 7 days after the inoculation.

and then used for preparation of inoculum (that is prepared in a similar way as spawn for the mushroom culture).

9. Incubation of the inoculated containers at 23–25°C during 1 month approximately, in obscurity, till deformation of a compact mycelium block (Fig. 2).

It is to the best intention of a researcher to check the content of investigated chemical species in native or commercial barley seeds so as to be sure that any contamination will not influence the experimental results. Therefore, samples of the substrate should be prepared. In case of the cesium transfer investigation, a routine analysis is done with an NaI or HPGe gamma spectrometer pointing to a high level of  $^{40}\text{K}$  but not  $^{137}\text{Cs}$  (data not shown).

### Induction of fructification (*ex vitro*)

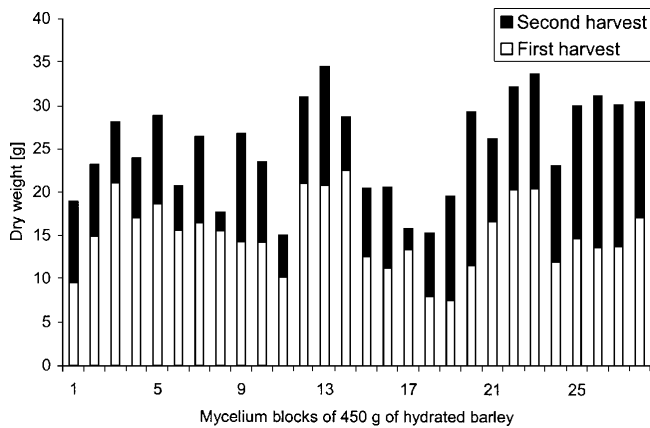
1. Removal of the cap of the plastic container and mechanical separation of the mycelium block and the plastic walls of the container – to improve development of the exchange interphase “liquid medium with radionuclides–mycelium–fruitbodies”.
2. Hydration of the block during 2 h and eliminating the excess of water.
3. Add to the block surface 3 mm of sterilized peat (120°C during 2 h) covering surface of the substrate.
4. Adequate hydration by spraying onto the peat surface (without presence of liquid water on the peat surface).
5. Incubation at the temperature of 18°C, with 500–1000 lux the light requirements and 90% relative humidity during the formation of primordia and 85% during the development and maturation.
6. Hydration several times a day of the peat surface by spraying without presence of liquid water on the peat surface but with liquid water in the lateral interphase.
7. Formation of primordia 7 days a week and, from now, increase of substrate hydration avoiding superficial water spotting till the development and maturation of the fructification next week.
8. Mature mushroom recollection (1st harvest) and generation of the 2nd harvest during 15 next days.

**Table 1.** Fresh and dry weight of the fruitbodies collected during the 1st and the 2nd harvest.

Samples	Fresh weight, g			Dry weight, g		
	1st harvest	2nd harvest	Total	1st harvest	2nd harvest	Total
Average	64.13	45.24	109.37	15.15	10.05	25.20
%	58.64*	41.36**		60.11*	39.89**	

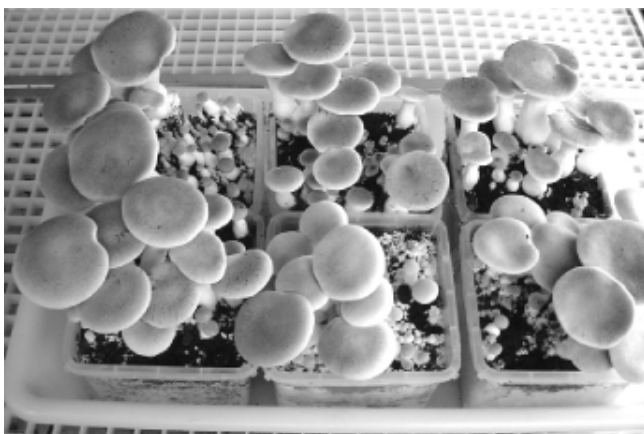
\* Fruiting bodies with less amount of water.

\*\* Fruiting bodies containing more water.

**Fig. 3.** The fructification (1st and 2nd harvest) of *Pleurotus eryngii* obtained from 28 mycelium blocks of 450 g of hydrated barley.

## Results and conclusions

Due to the presented productivity data (Fig. 3) and Table 1, one gets a greater productivity in the 1st harvest (Fig. 4). The average values were 64.13 g (1st harvest) and 45.24 g of biomass (2nd harvest) in each container with 450 g of hydrated barley. Moreover, the 1st harvest presents better quality which is corroborated by a greater dry weight yield in the 1st harvest (Table 1) comparing to the percent level of fresh and dry weight of the 1st and 2nd harvest. The 2nd harvest biomass is characterized by a greater content of water, as a result of the lower ability to produce mycelium to form fruitbodies above the block. The water relations have to be taken into account when applying radionuclides or heavy metals in the spawn/container interphase. The

**Fig. 4.** Fructification of *Pleurotus eryngii* (1st harvest).

3rd harvest was obtained only in several repetitions. The average number of mushrooms was 7 and 4.5 in the 1st and 2nd harvest, respectively (data not shown), pointing to a decrease in the mycelium potential to come out with its biomass but also the reduced ability to form primordia in the early phase of fructification.

The described procedure gives a possibility to carry out successful well-controlled laboratory cultures and apply them in various toxicological studies. The knowledge of substrate and exact culture conditions make the method reliable, reproducible and easy to modify for the individual research requirements.

**Acknowledgments** This project was financed by Vicerrectorado de Investigación, Universidad de Alcalá (PI2004/025). P.L.U. thanks the European Union for one-year “Socrates” fellowship in Spain.

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