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Preliminary study of platinum accumulation in the fruitbodies of a model fungal species: king oyster mushroom (*Pleurotus eryngii*)

Pawel L. Urban, Michał A. Bazała, Monika Asztemborska, José L. Manjón, Joanna Kowalska, Grażyna Bystrzejewska-Piotrowska, Dariusz Pianka, Romuald Stęborowski, Robert T. Kuthan

Abstract A model species of saprophytic fungus, king oyster mushroom (*Pleurotus eryngii*), was cultivated on barley substrate supplied with $[Pt(NH_3)_4](NO_3)_2$, under well defined conditions. The samples of the collected fruiting bodies were digested and analyzed for total platinum content by means of ICP-MS. The results proved that platinum is not accumulated in the fruitbodies of *Pleurotus eryngii* for a wide range of Pt concentrations in the culture substrate (100–1000 ppb Pt in 50 ml of water solution added to *ca*. 450 g of hydrated barley seeds per container). Observable levels of Pt were only found in the fruitbodies obtained from the medium contaminated with 10000 ppb (10 ppm) platinum solution. This demonstrates significant difference in the effectiveness of platinum extraction in fungi and plants, which are capable to accumulate platinum even when supplied at lower concentration (<500 ppb). It also shows different physiological pathways of platinum and other elements which are easily accumulated in the fruitbodies of the same species.

Key words bioaccumulation • bioremediation • fungi • heavy metals • mushrooms • mycoextraction • platinum • *Pleurotus eryngii*

P. L. Urban, M. A. Bazała,
G. Bystrzejewska-Piotrowska✉, D. Pianka,
R. Stęborowski
Isotope Laboratory,
Faculty of Biology, Warsaw University,
1 Miecznikowa Str., PL-02-096 Warsaw, Poland,
Tel.: +48 22-5542300, Fax: +48 22-5541106,
E-mail: byst@biol.uw.edu.pl

M. Asztemborska, J. Kowalska
Laboratory of Applied Analytical Chemistry,
Faculty of Chemistry, Warsaw University,
1 Pasteura Str., PL-02-093 Warsaw, Poland

J. L. Manjón
Departamento de Biología Vegetal,
Facultad de Biología, Universidad de Alcalá,
28871 Alcalá de Henares (Madrid),
Crtra. Madrid-Barcelona, Km. 33,600, Spain

R. T. Kuthan
Department of Medical Microbiology,
Medical University of Warsaw,
5 Chałubińskiego Str., PL-02-004 Warsaw, Poland

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Introduction

Studies on mycoextraction of environmental contaminants revealed a high ability of fungi to accumulate common pollutants present in the biosphere at trace levels, mainly heavy metals and radionuclides [8, 16, 20]. For a long time, conclusions could only be drawn from the analyses of environmental samples due to the communication gap that endured between analytical laboratories and practitioners of mushroom cultivation. An interest in accumulation of heavy metals in brown and white-rot fungi have been expressed in the reviews by Jellison [15] and Baldrian [3]. Application of fungi in bioremediation from such contaminants has also been peaked in the book edited by Gadd [12].

Unlike in the case of plants, culturing mushrooms in well-defined conditions is associated with numerous methodological difficulties. Hence, many studies of physiological features of fungi have been limited to their vegetative forms. Mycelial culture in a liquid medium does not reflect the real properties of fungi to take up elements as it is in nature [3]. Growing mushrooms in the well-defined and controlled conditions usually requires application of specialized techniques employing sterilized materials [24, 25]. Many basidiomycetes also require presence of certain microbial strains to induce fructification, excluding this way sterility of all the culture components used for cultivation. Recently, an effort has been made to adapt some known techniques of mushroom cultivation [21] to the studies of ecophy-

siological properties of mushrooms, i.e. their ability to take up and accumulate substances originating from chemical pollution of the natural environment [2, 14, 19, 26]. In spite of that, the spectrum of elements applied to the cultures of macromycetes has been limited to the most toxic ones, e.g. cadmium [5–7, 23], caesium [2, 14, 26], cobalt [22, 23], copper [22, 23], lead [22, 23], mercury [4–7, 22, 23], plutonium [14], selenium [26] and strontium [2, 14].

To date, different models have been used in the studies aiming at evaluation of fungal ability to accumulate the investigated elements, e.g.: *Agaricus bisporus* [26], *Agrocybe aegerita* [5, 7], *Pleurotus cornucopiae* [6], *Pleurotus eryngii* [2, 14], *Pleurotus ostreatus* [4], *Pleurotus sajor-caju* [23], *Volvariella volvacea* [22]. The culture of button mushrooms (*Agaricus* sp.) requires complex substrates, therefore it does not allow for sufficient control of the growing conditions. *Pleurotus* sp. seems to be an advantageous model. Although culturing oyster mushroom (*Pleurotus ostreatus*) is straightforward, this species does not prove to be a suitable model for ecophysiological studies. It usually grows on alive or dead tree trunks, thus it does not exhibit the features common for the vast number of mushrooms appearing above the soil surface. A less known species, *Pleurotus eryngii*, is a typical grassland saprophyte in the Mediterranean zone, and already it has successfully been employed for the studies of accumulation of radionuclides [2, 19]. Therefore, this species has also been chosen for the purpose of this study.

Platinum is an element to which environmental and analytical chemists have recently paid much attention [13, 17, 18]. Apart from natural sources, it gets to the soil in the form of particles released from the automotive exhaust converters which are dispersed in the atmosphere. Its toxicity has been proven during the study employing a rat model showing Pt accumulation in many organs after inhalation by the animals [1]. Several plant species (e.g. mustard and maize) were shown to accumulate platinum to a great extent [17, 18]. Although platinum concentration in wild fungi does not provide any cause for concern for individuals eating these foods [11], it may become a problem in the future. The analyses of environmental samples revealed a low concentration of platinum in a saprophytic mushroom *Vascellum pratense*, collected from the vicinity of German highways [10].

Due to absence of data on the physiological ability of mushrooms to take up and accumulate platinum, the aim of this work is to assess the intensity of Pt accumulation in fruitbodies of the model fungus (*Pleurotus eryngii*), excluding influence of the soil composition and other environmental conditions on bioavailability of the supplied platinum complex.

Materials and methods

Chemicals and disposables

Nitric, hydrochloric and perchloric acids were from Merck. The platinum complex $[Pt(NH_3)_4](NO_3)_2$ was from Sigma Aldrich. All the chemicals were of the highest analytical grade.

The seeds of a brewing cultivar of barley (*Hordeum vulgare* L.), obtained from suppliers in Guadalajara (Spain) and Ożarów Mazowiecki (Poland), were used as the culture substrate. The containers for *in vitro* tissue cultures were obtained from Duchefa (The Netherlands).

Analytical equipment

The laboratory dryer was from Memmert (Germany). The laboratory mill, from Fritsch (Germany), was used for homogenization of the organic material. A domestic microwave oven, Sharp, R-5A51, 850 W (Japan) together with low-pressure vessels (max. pressure 13.8 bar), type P/N 323000, CEM (USA), were used for the sample digestions. The Inductively Coupled Plasma Mass Spectrometer, SCIEX "Elan 6100 DRC" was from Perkin Elmer (USA). Distilled water used throughout the study was additionally purified with a Milli-Q system to reach the resistivity >16 MΩ m.

Mushroom cultivation

The strain of king oyster mushroom *Pleurotus eryngii* (De Candolle ex Fries) Quelet sensu lato (AHMD-401) was isolated from a fruitbody found growing on the remains of roots of an *Eryngium campestre* L. plant found in the locality of Camarma de Esteruelas (3° 22' 45" W, 40° 33' 15" N, 30T 4684489 UTM; Madrid Province, Spain). Then, it was cultured on 2% malt agar medium in the autumn of 1995, and stored at 4°C.

The Pt solutions were prepared by dissolving appropriate amount of $[Pt(NH_3)_4](NO_3)_2$ in distilled water (with addition of HNO_3), to obtain the final concentration of 100, 500, 2000, 4000 and 10000 ppb Pt. Pure distilled water was added to the pots serving as a control. The 1st experiment, carried out in the period April–June 2004, involved the concentrations 500, 2000 and 4000 ppb Pt. The 2nd one, May–July 2005, was designed to prove accumulation at the low and high concentration extremes, 100 and 10000 ppb, respectively.

Several procedures for cultivation of *Pleurotus eryngii* are described in the literature [19, 21, 24]. Briefly, barley seeds are washed and hydrated in water for 24 h. After soaking, 450 g of hydrated barley is placed in the *in vitro* cultivation container. 50 ml of platinum solution is spread over the surface. After 3 h, the containers are autoclaved at 130°C for 100 min. The mycelium substrate was placed on the surface of barley under laminar air flow hood. The containers were left at 25°C in darkness untill the hyphae have covered the whole of barley seeds. They were uncovered and 20 g of sterile peat soil was spread over the spawn surface. Then, they were transferred to the plant growing chambers equipped with temperature, light and humidity control systems together with two independent humidifiers (20°C, 16 h photoperiod, humidity 80–85%). Formation of primordia was improved by irradiation with a UV lamp for 3 h. In the 2nd experiment, a growth chamber made of expanded polystyrene was applied to maintain satisfactory humidity (Fig. 1). The culture was moisturized by spraying water over the containers at least once a day

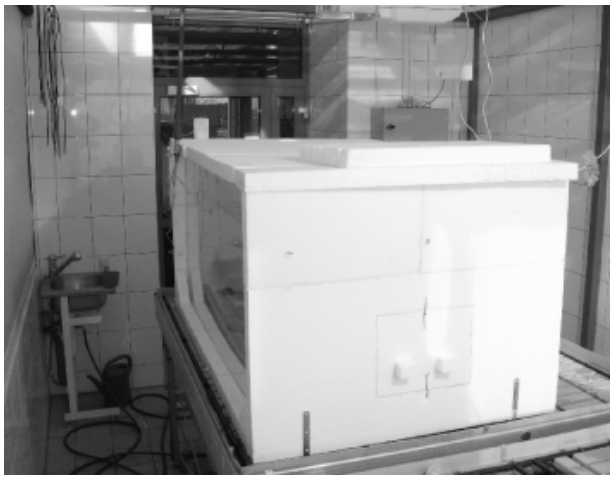

Fig. 1. The expanded polystyrene chamber constructed inside the greenhouse to maintain high humidity necessary for the developement of fruitbodies.

(1st experiment) or by using a moisturizer (2nd experiment), which also decreased infections with moulds. After reaching maturity, the fruitbodies were collected, by cutting the base with a stainless steel blade, then weighed and dried at 105°C until total dehydration. Up to three harvests of the fruitbodies could be done.

Analysis of platinum

The protocol for analysis of Pt in plant tissues using ICP-MS and voltammetry was described by Kowalska *et al*. [18] and it was followed for the fungal samples in its major steps. Dry samples were homogenized in the mill during 1 h (100 rev min^{-1}). *Ca*. 250 mg of each sample was weighed and put in the CEM teflon vessel followed by adding 1 ml HNO_3, 1 ml $HClO_4$ and 0.5 ml HCl. The vessels were screwed up tightly and placed in the microwave oven. Heating the samples was carried out in several cycles by increasing their duration as well as power of microwaves according to the following sequence: 3 min 255 W, 4 min 425 W, 5 min 595 W, 6 min 850 W, with 2 min break between the cycles. After cooling down, the digests were quantitatively transferred into volumetric flasks and adjusted to 25 ml with distilled water.

ICP-MS measurements were carried out applying the following settings: sweep 5, replicates 5, dwell time 0.1 s, ICP RF power 1100 W, lens voltage 7 V, nebulizer gas flow 0.98 $l \cdot min^{-1}$, plasma gas flow 15 $l \cdot min^{-1}$. The measured isotope was ^{195}Pt. Quantitative Analysis Program automatically corrected the intensities for any interferences originating from isobaric and molecular ions. The calibration curve method was applied for quantitative determinations.

Results and conclusions

Mature fruitbodies were collected from the both culture lots (Fig. 2). The ICP-MS measurements proved that Pt concentrations in the prepared samples of fruitbodies, obtained from the media contaminated with Pt solutions up to 4000 ppb, were below the limit of

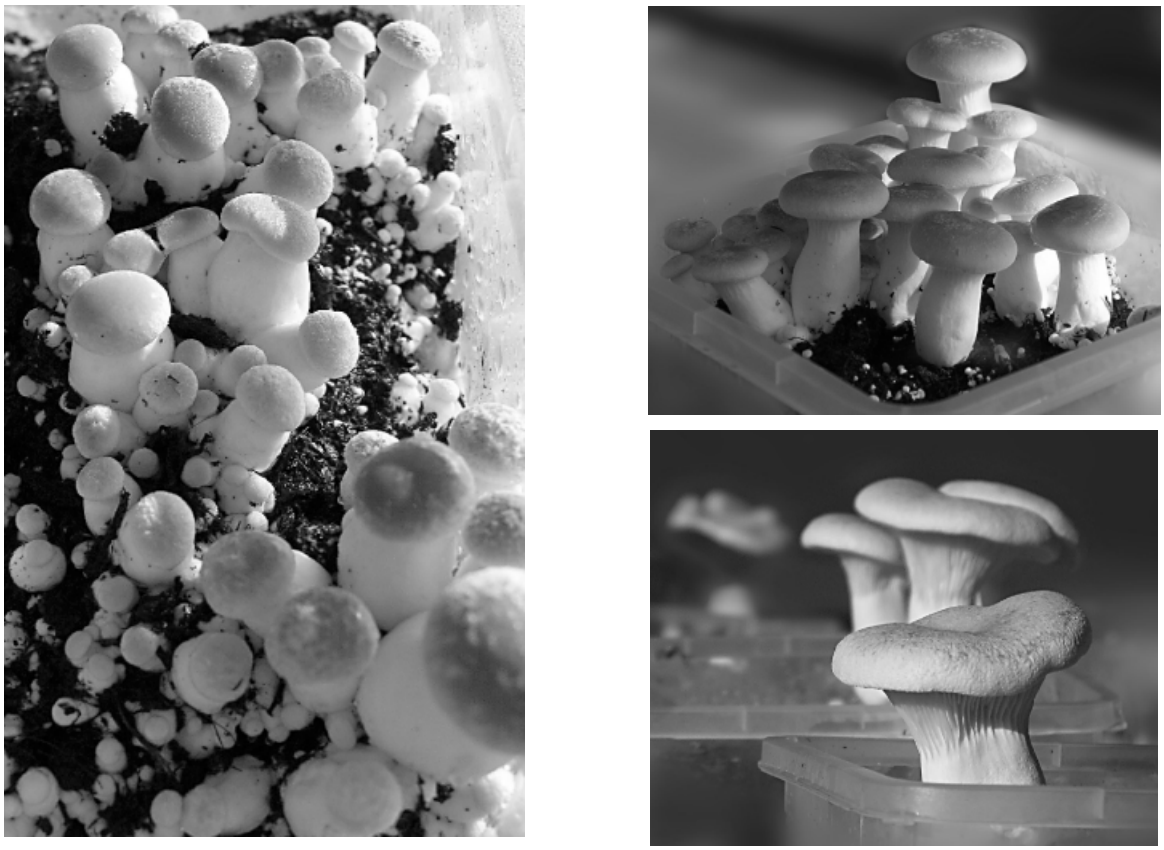

Fig. 2. Fruitbodies of *Pleurotus eryngii* in various stages of development.

Table 1. Platinum concentration in the substrate (variants with 100 and 10000 ppb) and the fruitbodies of *Pleurotus eryngii*. The detection limit DL of the method was 8.12 ng·g^{-1}

Added Pt	Sample	Pt content [ng·g^{-1} d.w.]	SD
100 ppb	medium	9.26	0.74
	caps	<DL	–
	stipes	<DL	–
10000 ppb	medium	931	19
	caps	88.3	1.4
	stipes	69.2	2.6

detection of the applied analytical procedure (Table 1). Only fruitbodies collected from the medium with the highest Pt concentration (10 ppm Pt in 50 ml solution per pot) contained a detectable amount of Pt (>40 ng Pt g^{-1} d.w.). Platinum was also found in the substrate block coated with fungal hyphae (~930 ng Pt g^{-1} d.w. for the 10 ppm variant).

Until now, scarce data on the fate of platinum in mushrooms could be found [10]. The obtained result is surprising and shows low capability of the saprophytic mushrooms to take up platinum. It points to entirely different behaviour of platinum in fungi than it is observed in plants which often take up platinum to a high extent [17, 18]. Moreover, other heavy metals are accumulated in fruitbodies of many basidiomycetes to a great degree [3, 15].

The concentration of platinum, determined in the mushrooms grown on 10 ppm Pt-contaminated medium is still lower than that found in plants grown in hydroponics with two orders of magnitude lower Pt concentrations.

Recently, it was suggested that, in nature, translocation of substances via mycelium hyphae normally occurs on shorter distances, usually <75 cm, reaching velocities up to a few cm·h^{-1} [9]. Therefore, to enhance effectiveness of bioremediation techniques, a close contact of large amounts of mycelium with the contamination carrier needs to exist. However, as the present result show, that in commonly occuring Pt pollution platinum cannot be transported in the mycelium even over short distances. The reason for that may be complexation of Pt in the polysaccharide-rich hyphal cell wall as well as by peptide ligands inside the cells. The conclusive results can be achieved by applying fractionation and speciation analysis.

The effectiveness of mycoextraction seems to differ for various chemical species since accumulation of platinum is much less intense than that of other contaminants. The study shows that the saprophytic fungi are not good candidates to be used in bioindication nor bioremediation.

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References

1. Artelt S, Creutzenberg O, Kock H *et al.* (1999) Bioavailability of fine dispersed platinum as emitted from automotive catalytic converters: a model study. Sci Total Environ 228:219–242
2. Baeza A, Guillén J, Paniagua JM *et al.* (2000) Radiocaesium and radiostrontium uptake by fruit bodies of *Pleurotus eryngii* via mycelium, soil and aerial absorption. Appl Radiat Isot 53:455–462
3. Baldrian P (2003) Interactions of heavy metals with white-rot fungi. Enz Microb Technol 32:78–91
4. Bressa G, Cima L, Costa P (1988) Bioaccumulation of Hg in the mushroom *Pleurotus ostreatus*. Ecotox Environ Safety 16:85–89
5. Brunnert H, Zadražil F (1979) The cycling of cadmium and mercury between substrate and fruiting bodies of *Agrocybe aegerita* (a fungal model system). Eur J Appl Microbiol Biotechnol 6:389–395
6. Brunnert H, Zadražil F (1980) Translation of cadmium and mercury in straw columns colonized by the fungus *Pleurotus cornucopiae* Paul ex Fr. Eur J Appl Microbiol Biotechnol 10:145–154
7. Brunnert H, Zadražil F (1981) Translation of cadmium and mercury into the fruiting bodies of *Agrocybe aegerita* in a model system using agar platelets as substrate. Eur J Appl Microbiol Biotechnol 12:179–182
8. Bystrzejewska-Piotrowska G, Urban PL, Stęborowski R (2003) Discrimination between ^{137}Cs and ^{40}K in the fruiting body of wild edible mushrooms. Nukleonika 48:155–157
9. Cairney JWG (2005) Basidiomycete mycelia in forest soils: dimensions, dynamics and roles in nutrient distribution. Mycol Res 109:7–20
10. Djingova R, Kovacheva P, Wagner G, Markert B (2003) Distribution of platinum group elements and other traffic related elements among different plants along some highways in Germany. Sci Total Environ 308:235–246
11. Food Standards Agency (2000) MAFF UK – Multi-element survey of wild edible fungi and blackberries (Sheet 199), http://www.food.gov.uk/science/surveillance/maffinfo/2000/maff-2000-199
12. Gadd GM (ed.) (2001) Fungi in bioremediation. British Mycological Society, Cambridge University Press, Cambridge
13. Godlewska-Żyłkiewicz B (2003) Biosorption of platinum and palladium for their separation/preconcentration prior to graphite furnace atomic absorption spectrometric determination. Spectrochim Acta B 58:1531–1540
14. Guillén Gerada FJ (2002) Estudio de la transferencia dela contaminación radioactiva a los hongos. PhD Thesis, Universidad de Extremadura
15. Jellison J, Connolly J, Goodell B *et al.* (1997) The role of cations in the biodegradation of wood by the brown-rot fungi. Int Biodeterior Biodegrad 39:165–179
16. Kalač P, Svoboda L (2000) A review of trace element concentrations in edible mushrooms. Food Chem 69:273–281
17. Kowalska J, Asztemborska M, Bystrzejewska-Piotrowska G (2004) Platinum uptake by mustard (*Sinapis alba* L.) and maize (*Zea mays* L.) plants. Nukleonika 49;S1:S31–S34
18. Kowalska J, Huszał S, Sawicki MG *et al.* (2004) Voltammetric determination of platinum in plant material. Electroanal 15:1266–1270
19. Manjón JL, Urban PL, Bystrzejewska-Piotrowska G (2004) A simple and quick model to study uptake and transfer of radionuclides and heavy metals from mycelium to the fruitbody of saprophytic edible fungi. Nukleonika 49;S1:S21–S24

20. Mietelski JW, Jasińska M, Kubica B, Kozak K, Macharski P (1994) Radioactive contamination of Polish mushrooms. Sci Total Environ 157:217–226
21. Pérez MV, Estrada R, García-Montero LG, Lizarraga M, Manjón JL (1996) Estudios preliminares sobre el cultivo en invernaderos de *Pleurotus eryngii* (DC.:Fr.). Quél Boletín de la Sociedad Micológica de Madrid 21:401–404
22. Purkayastha RP, Mitra AK (1992) Metal uptake by mycelia during submerged growth and by sporocarps of an edible fungus *Volvariella volvacea*. Indian J Exp Biol 30:1184–1187
23. Purkayastha RP, Mitra AK, Bhattacharyya B (1994) Uptake and toxicological effects of some heavy metals on *Pleurotus sajor-caju* (Fr.) Singer. Ecotox Environ Safety 27:7–13
24. Stamets P (2000) Growing gourmet and medicinal mushrooms. Ten Speed Press, Berkeley
25. Stamets P, Chilton JS (1983) The mushroom cultivator. A practical guide to growing mushrooms at home. Agarikon Press, Washington
26. van Elteren JT, Woroniecka UD, Kroon KJ (1998) Accumulation and distribution of selenium and cesium in the cultivated mushroom *Agaricus bisporus* – a radiotracer-aided study. Chemosphere 36:1787–1798