Cadmium and manganese in contrast to calcium reduce yield and nutritional values of the edible mushroom *Pleurotus pulmonarius*

S. W. CHIU[1], Y. H. CHAN[1], S. C. LAW[1], K. T. CHEUNG[1] AND D. MOORE[2]

[1] *Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong*
[2] *School of Biological Sciences, The University of Manchester, Manchester M13 9PT, U.K.*

Pleurotus pulmonarius is a species of the oyster mushroom which has become the second most popularly cultivated mushroom in the world. In this study, we show that renewed fruiting from excised stipes can be used as a simple and rapid *in vitro* bioassay system to detect fruiting modulators. We used this and conventional cultivation techniques to examine the effects of cadmium, a potential contaminant from industrial sources, calcium, which is an ingredient in mushroom compost, and manganese, which has been claimed to improve the yield of *P. ostreatus*. All the three metallic salts did not affect sporulation. Calcium chloride addition shortened the time taken for the mushroom to fully cover the cultivation compost and improved yield. Insoluble calcium salts at higher concentrations had a similar though less pronounced effect. The calcium and total amino acid contents of fruit bodies also increased. Compost supplementation with calcium is desirable for cultivation of the oyster mushroom but not indispensable since the straw-based cultivation substrate is itself able to provide the required minerals. By contrast, manganese chloride retarded mycelial growth and decreased yield but increased the total amino acid content in the stipe whilst manganese sulphate did not enhance accumulation of manganese into fruit bodies. Excess manganese induced browning of vegetative tissues. Cadmium ions did not kill the oyster mushroom at 4·5 mM but reduced yield by 50%. At this concentration cadmium decreased the total amino acid content and affected the amino acid profile but did not affect the form and shape of the fruit bodies. *Pleurotus pulmonarius* concentrated cadmium to such an extent that consumption of as little as 20 g (D.W.) of the most contaminated samples would exceed the weekly limit tolerated by humans and thus pose a health hazard. Monitoring the heavy metal contents of mushrooms marketed for food is, therefore, advised as the source of the substrate for cultivation is usually not known.

Pleurotus species fruit on a wide variety of solid lignocellulosic substrates (Mueller *et al*., 1985; Bisara *et al*., 1987; Ragunathan *et al*., 1996), and this genus has become one of the most popularly cultivated mushrooms in the world second to *Agaricus* (Royse, 1995). These mushrooms are in the Lentinaceae, characterized by bell-shaped fruit bodies bearing a gill hymenophore, with *P. ostreatus* commonly cultivated in Europe and *P. sajor-caju* (some of which are actually *P. pulmonarius*) in tropical countries (Bresinsky *et al*., 1987; Petersen & Hughes, 1993; Iracabal *et al*., 1995; Zervakis & Balis, 1996). Mushroom cultivation has long been a bio-conversion process and simultaneously a useful approach to treating solid agricultural and industrial wastes in both developed and developing countries (Chang & Chiu, 1992; Levanon, 1993). In cultivation, insoluble calcium salts are added (i) to increase pH to neutrality and thereby probably reducing the bacterial contamination, and (ii) to increase aeration by aggregation, improving the texture and porosity of the compost (Bech & Rasmussen, 1968; Hayes, 1972). Calcium is known to stimulate fruiting in *A. bisporus* (Hayes, 1972) but to inhibit it in *Lentinula edodes* (Leatham & Stahmann, 1989). Recently, irrigation water supplemented with calcium chloride has been shown to improve post-harvest storage by reducing the surface bacterial population. This treatment also enhances whiteness in *A. bisporus* (Beelman *et al*., 1993; Miklus & Beelman, 1996).

Manganese chloride has also been added to improve the yield of *P. ostreatus* (Lelley & Jann, 1993). Manganese regulates many enzymic activities (Hughes & Poole, 1989; Auling, 1994; Keen *et al*., 1994), and exogenous manganese stimulates lignin degradation by *P. ostreatus* and *P. pulmonarius* (Tsang *et al*., 1987; Kerem & Hadar, 1993; Camarero *et al*., 1996). The latter is important because the fungus must degrade this component to support growth and fruiting. On the other hand, high concentrations of manganese chloride have been used to induce mutation and to inhibit growth in yeast, *Saccharomyces cerevisiae*, and *P. ostreatus* (Auling, 1994; Matsumoto & Fukumasa-Nakai, 1994; Farcasanu *et al*., 1996). Manganese toxicity is pH-dependent and species-specific (Babich & Stotzky, 1981).

In contrast to the beneficial effects of some metals, macrofungi, including edible mushrooms, have long been known to accumulate toxic or radioactive metals from the natural and artificial environment (Tyler, 1980; Brunnert & Zadražil, 1983; Jain *et al*., 1989; Lepsova & Mejstrik, 1989; Sanglimsuwan *et al*., 1993; Gabriel *et al*., 1994; Purkayastha *et*

al., 1994; McDougall & Blanchette, 1996). Intake of cadmium-contaminated food, which is the major route of cadmium poisoning (Tathvonen, 1996), causes renal dysfunction, hepatic injury and skeletal disorders (Piscator, 1988; Waalkes & Oberdorster, 1990). Cadmium binds to the sulphydryl groups of essential enzymes and to phospholipids and nucleic acids, and can interfere with oxidative phosphorylation (Piscator, 1988). Cadmium ions are also mutagenic, altering DNA polymerase fidelity, stimulating putative RNA synthesis and disturbing DNA repair mechanisms (Piscator, 1988; Frank *et al*., 1994). Increased cadmium emissions from industrial production and waste disposal, combined with long-term persistence of cadmium in the environment, and relatively rapid uptake and accumulation by food chain crops contribute to its potentially hazardous nature. The relevance of this to oyster mushroom cultivation is the possibility that contaminated substrates might be used for cultivation by accident. In many third-world countries, city planning has not been emphasized, and industries and agricultural land are scattered and intermingled. Incidences of contaminated mushroom products sold in the market have been reported (Vetter, 1994; Haldimann *et al*., 1995). It is important, therefore, to determine if there are any observable symptoms associated with heavy metal-contaminated fruit bodies so that the contaminated crop can be removed from the market or avoided. This study examines the effects of calcium, manganese and cadmium salts on the cultivation, fruiting and sporulation, and nutritive contents of *P. pulmonarius*.

MATERIALS AND METHODS

Mushroom cultivation

Pleurotus pulmonarius Fr. (= *P. sajor-caju* (Fr.) Singer) strain P127, an Indian commercial strain from Dr Zakia Bano (Central Food Technology, Research Institute, Mysore, India), was used in this study and maintained on potato dextrose agar (PDA) at 25 °C. Seven-day-old mycelium grown in PD broth was harvested and homogenized with a Waring blender. Fixed inoculum of 3 ml of 0·08 g wet wt mycelium ml^{-1} distilled water was inoculated onto compost. The compost comprised 18% straw, 2% wheat bran, and 80% water. One of the following salts was added to the final concentrations of the compost: calcium chloride (up to 200 mM), calcium carbonate (up to 625 mM (= 5%)), calcium hydroxide (up to 2·2 M (= 13%)), manganese chloride (up to 40 mM), manganese sulphate (up to 40 mM), and calcium chloride (up to 4·5 mM). Cadmium chloride was also added to wastepaper-based compost (wastepaper replaced straw in the compost), and in this case, 1% (= 169 mM) calcium hydroxide was added as a supplement. After thorough mixing, the compost would be fermented for 2 d before packing into autoclavable plastic bags. Five bags each weighing 250 g were used for each treatment. After the complete colonization of compost with mycelia, the bags were transferred to an environmental chamber kept at 25° with 80% r.h. under 8 h light/16 h dark illumination cycle. One week later, mature fruit bodies with the pileus fully expanded were harvested and weighed before lyophilization.

Renewed fruiting in excised stipes

Chiu & To (1993) found that excised stipes of *P. pulmonarius* could renew fruiting even in water agar as reported for *Coprinus cinereus* (Chiu & Moore, 1988). The basal portion of the stipe of a mature fruit body was chopped into 1 cm lengths using aseptic technique. The segments were then placed on buffered agar (pH 6·8) which consisted of: 2% agar (bacteriological grade, Difco), 1 mM EDTA (dipotassium salt), and 10 mM PIPES (dipotassium salt) (Good *et al*., 1966). Calcium was tested at concentrations up to 100 mM (carbonate and hydroxide are insoluble but were added at 100 mM equivalent amount, respectively). The effects of manganese salts (chloride and sulphate) were tested at concentrations up to 40 mM, and cadmium chloride was added at up to 545 μM. A total of 60 samples per treatment was tested. The inoculated plates were incubated at 28° in darkness and the frequency of renewed fruiting (new primordia formed directly on the excised stipes) was recorded.

Chemical analyses

The freeze-dried mushrooms were ground into powder of 0·2 mm diam. using a grinder (Cyclotec (Tecator) 1093 sample mill, France), followed by acid hydrolysis to determine the amino acid composition using an amino acid analyser (Beckman 7300 system, CA, U.S.A.). The preparation follows the manufacturer's instructions and Gehrke *et al*. (1985). Three replicates were analysed for each test. For metal determination, all glassware and plasticware were acid-treated prior to use. After ashing and dissolving the metals from the ash with 6 N hydrochloric acid according to AOAC (1990), the metal contents were determined with inductively coupled plasma spectrometry (Atomscan 16 sequential Plasma Spectrometer, Thermo Jarrell Ash, MA, U.S.A.) or with atomic absorption spectrophotometry (Hitachi, model Z-8200 series polarized Zeeman atomic absorption spectrophotometer, Japan). Five replicates were analysed for each test.

The C, N, H and S contents of the fruit bodies were determined with 2 mg of fruit body powder (accuracy to 1 μg with AD-4 autobalance, Perkin Elmer, NJ, U.S.A.) and placed into the CHNS/O analyser (PE 2400, Perkin Elmer, NJ, U.S.A.). Five replicates were analysed for each test. All the data were treated with ANOVA and Duncan multiple range test using a significance level of 0·05.

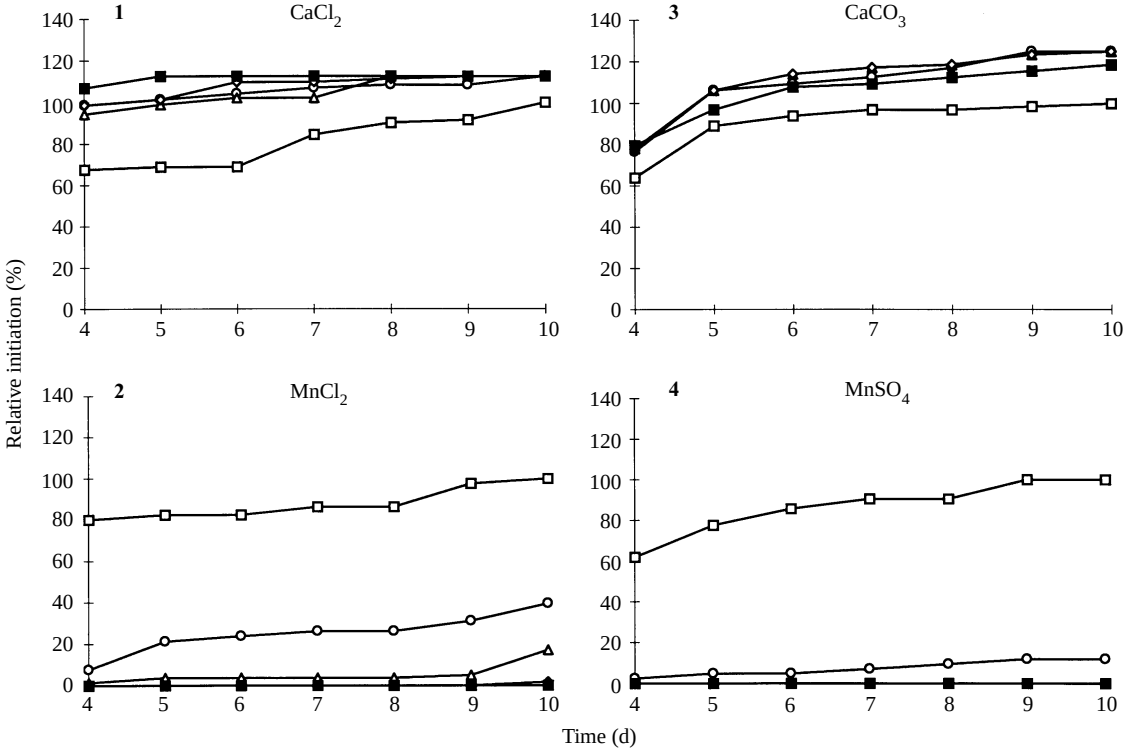
Scanning electron microscopy

Specimens were freeze-dried, coated with carbon and scanned with a Cambridge 360 electron microscope (Oxford, U.K.) equipped with a Link Analytical X-ray microanalyser (Oxford, U.K.) at 20 keV for 100 s. Afterwards, the specimen was further coated with gold before scanning for detection of sporulation (Chiu & Moore, 1990; Chiu & Poon, 1993).

RESULTS

Renewed fruiting, crop yield and growth rate

Both renewed fruiting and crop yield were expressed in relative terms to the control without addition of a metallic salt



Figs 1–4. Effects of metallic salts on renewed fruiting of excised stipes of *Pleurotus pulmonarius* strain P127. Sixty stipe segments were inoculated onto media supplemented with different amounts of metallic salts. **Fig. 1.** $CaCl_2$. The symbols denote: □, 0 mM; ○, 25 mM; △, 50 mM; ◇, 75 mM; ■, 100 mM. **Fig. 2.** $MnCl_2$. The symbols denote: □, 0 mM; ○, 10 mM; △, 20 mM; ◇, 30 mM; ■, 40 mM. **Fig. 3.** $CaCO_3$. The symbols denote: □, 0 mM; ○, 31·25 mM; △, 62·5 mM; ◇, 93·75 mM; ■, 125 mM. **Fig. 4.** $MnSO_4$. From 20 mM concentration onwards, no renewed fruiting was observed. The symbols denote: □, 0 mM; ○, 10 mM; △, 20 mM; ◇, 30 mM; ■, 40 mM. The number of stipe segments showing renewed fruiting at the zero concentration of a metallic salt is taken as 100%.

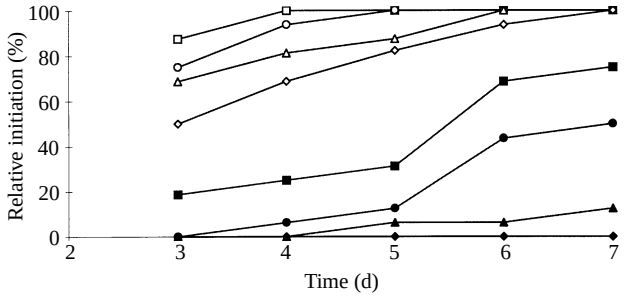


Fig. 5. Effect of $CdCl_2$ on renewed fruiting of excised stipes of *Pleurotus pulmonarius* strain P127. The symbols denote: □, 0 μM; ○, 16 μM; △, 27 μM; ◇, 54 μM; ■, 109 μM; ●, 218 μM; ▲, 436 μM; ◆, 545 μM. Conditions are set as in Figs 1–4. The number of stipe segments showing renewed fruiting at the zero concentration of a metallic salt is taken as 100%.

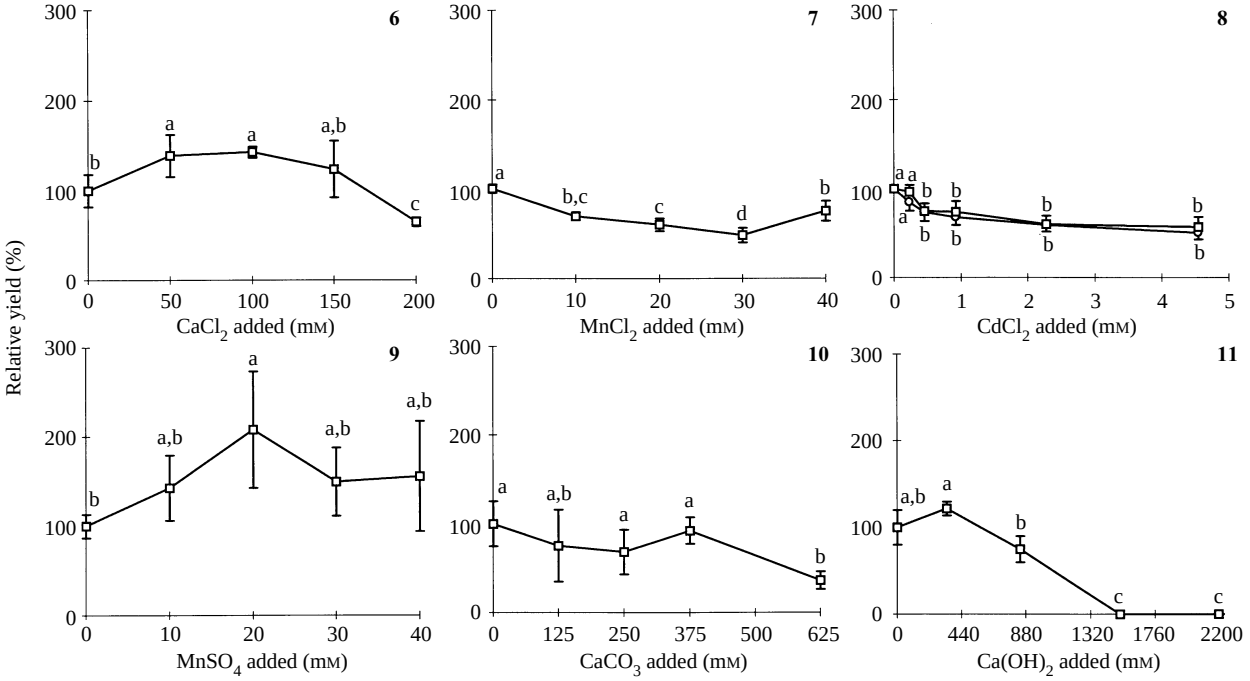
as the data obtained varied with the physiological age of the fungus. This makes cross-comparison eligible. For renewed fruiting, the presence of $CaCl_2$ or $CaCO_3$ enhanced fruiting initiation (Figs 1–4); maximum stimulation was obtained at or above 50 mM. In contrast, the presence of $MnCl_2$ at 10 and 20 mM and $MnSO_4$ at 10 mM delayed fruiting (Figs 2, 4). The higher concentrations of manganese salts led to the total inhibition of renewed fruiting, and a brown colour appeared surrounding the inoculum. The presence of $CdCl_2$ (at or above 16 μM) retarded fruiting, and total inhibition occurred at 545 μM (Fig. 5).

Table 1 shows the minimum time taken for the mushroom to completely cover the compost supplemented with a metallic salt. The results indicate that the manganese salts slowed down the growth rate while cadmium chloride did not have a serious effect. In contrast, $CaCl_2$ stimulated mycelial growth. In terms of relative yield (Figs 6–11), at concentrations below 150 mM for $CaCl_2$ and below 3% for insoluble calcium salts (500 mM for hydroxide and 375 mM for carbonate), yield was either stimulated or unaffected (Figs 6, 10, 11). Higher concentrations of calcium hydroxide reduced or completely inhibited the formation of fruit bodies, and the pH of the compost was increased to pH 9. Although manganese sulphate stimulated yield, variation among samples (as revealed by the standard deviation bar in Fig. 9) was very large. The presence of $MnCl_2$ reduced the yield (Fig. 7). Also, browning of tissues was found in manganese-supplemented compost. Similarly,

Table 1. The effect of metallic salts on the time taken for *Pleurotus pulmonarius* strain P127 to cover the mushroom compost

Metallic salt added	$CaCl_2$	$CaCO_3$	$Ca(OH)_2$	$MnCl_2$	$MnSO_4$	$CdCl_2$
Time (d)*	15	21	28	44	35	28

* The minimum time taken for the fungus to completely cover the metal-supplemented compost at one of the tested concentrations.



Figs 6–11. Effects of metallic salts on relative yield of fruit bodies of *Pleurotus pulmonarius* strain P127. **Fig. 6**, $CaCl_2$. **Fig. 7.** $MnCl_2$. **Fig. 8.** $CdCl_2$. □, straw compost; ○, waste paper compost. **Fig. 9.** $MnSO_4$. **Fig. 10.** $CaCo_3$. **Fig. 11.** $Ca(OH)_2$. The yield at the zero concentration of a metallic salt is taken as 100%. The data are presented as the mean of five replicates and S.D. Letters indicate the ranking after analysis with ANOVA and Duncan multiple range test. Rank 'a' is the greatest.

Table 2. The effects of metallic salts on the hydrogen, sulphur and nitrogen contents of fruit bodies of *Pleurotus pulmonarius* strain P127

	mM	H (%)	N (%)
$CaCl_2$ on stipe	0	7·59 ± 0·34 a	3·64 ± 0·58 a
	50	6·94 ± 0·17 b	1·82 ± 0·27 b
	100	6·84 ± 0·13 b	1·87 ± 0·46 b
	150	6·96 ± 0·11 b	1·50 ± 0·34 b
	200	7·07 ± 0·11 b	1·29 ± 0·24 b
	mM	S content in pileus (%)	S content in stipe (%)
$MnCl_2$	0	0·37 ± 0·07 b	0·92 ± 0·10 a
	10	0·60 ± 0·03 a	0·70 ± 0·03 b, c
	20	0·59 ± 0·01 a	0·76 ± 0·03 b
	30	0·58 ± 0·11 a, b	0·63 ± 0·04 c
	40	0·56 ± 0·05 a	0·65 ± 0·05 c
$CaCO_3$	0	1·03 ± 0·05 a	1·22 ± 0·07 a
	125	1·08 ± 0·09 a	1·10 ± 0·29 a, b
	250	0·85 ± 0·11 a, b	0·95 ± 0·07 b
	375	0·78 ± 0·10 a, b	0·88 ± 0·06 b
	625	0·65 ± 0·05 b	0·73 ± 0·09 b

Data are means of five replicates ± S.D. Letters indicate the ranking by Duncan multiple range test. Rank 'a' is the greatest.

the addition of calcium chloride to both types of compost (wastepaper and straw) reduced yields by 50% (Fig. 8).

CHNS contents

The carbon content was not affected by any treatments tested. The nitrogen and hydrogen contents decreased in the stipe only following the addition of $CaCl_2$ (Table 2). Addition of $CaCO_3$ or $MnCl_2$ reduced the S content of the stipe tissue (Table 2). While $CaCO_3$ also reduced the S content of the pileus, $MnCl_2$ addition resulted in an increase (Table 2), although the change in concentration was small. Finally, the presence of $CdCl_2$ did not affect the C, H and N contents (the S content was not determined).

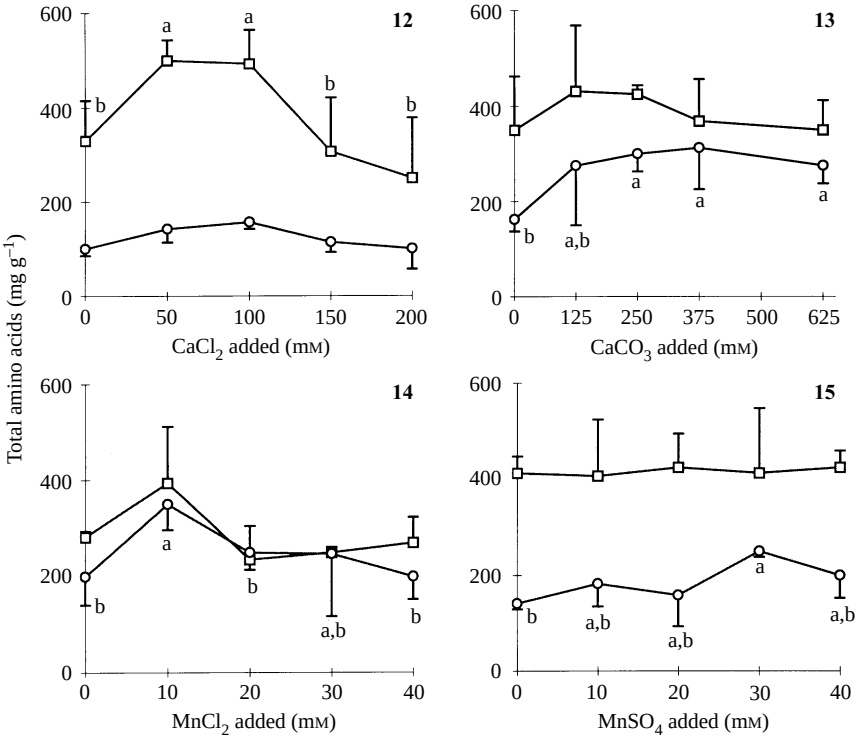
Amino acid contents

Except with $MnCl_2$, the total amino acid content was higher in the pileus than in the stipe (Figs 12–15). Calcium chloride (below 150 mM) increased the content in the pileus whereas $CaCO_3$ above 1% (= 125 mM) increased the total amino acid content of the stipe (Fig. 13). The two manganese salts slightly increased the total amino acid content only in the stipe (Figs 14–15), whilst the addition of 4·5 mM cadmium chloride decreased the total amino acid content by 11–17%.

In terms of individual amino acids, the S-containing amino acids (Cys and Met) were in most cases undetectable or present in trace amounts even from the fruit bodies harvested from compost supplemented with $MnSO_4$. For both manganese and calcium treatments, the amino acid profiles did not change. In contrast, $CdCl_2$ not only decreased the total amino acid content in both types of compost (wastepaper and straw) but also modified the amino acid profiles; the contents of the amino acids Thr, Ser and Arg decreased while those of Ala and Ile increased (Fig. 16).

Accumulation of metals

The cultivation substrates excluding the metallic salts to be added were rich in aluminium and contained trace amounts of



Figs 12–15. Effects of metallic salts on total amino acid contents of *Pleurotus pulmonarius* strain P127. **Fig. 12.** $CaCl_2$. **Fig. 13.** $CaCO_3$. **Fig. 14.** $MnCl_2$. **Fig. 15.** $MnSO_4$. □, pileus, ○, stipe. The data are presented as a mean of three replicates and S.D. Letters indicate the ranking after analysis with ANOVA and Duncan multiple range test. Rank 'a' is the greatest.

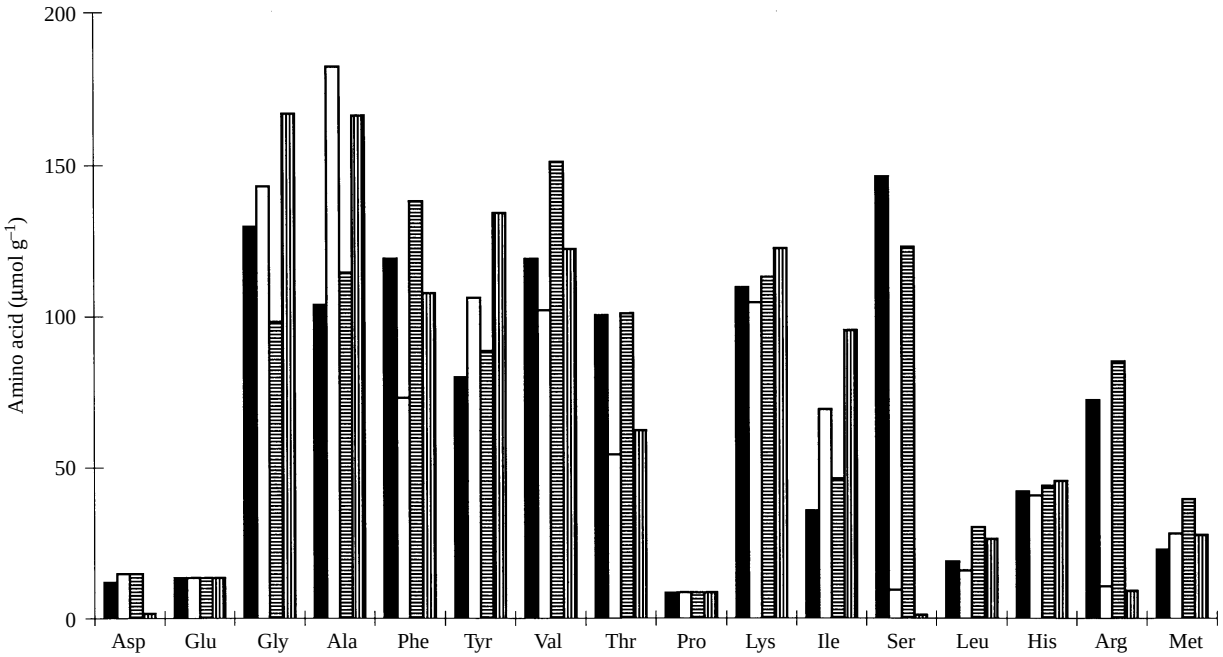


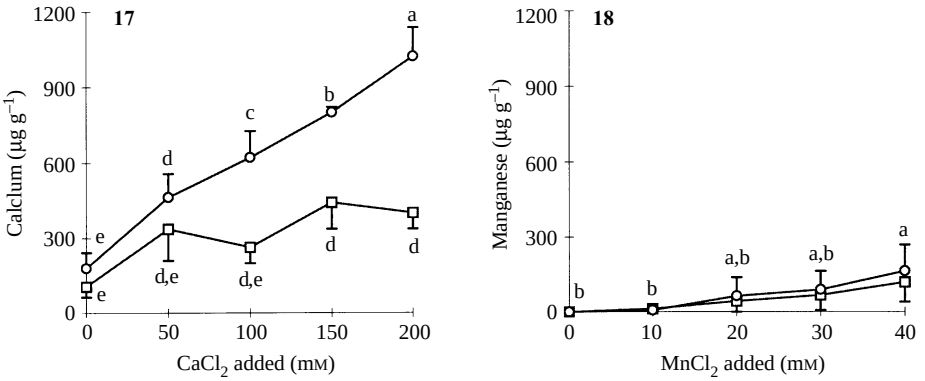
Fig. 16. Effect of $CdCl_2$ on the amino acid profiles of fruit bodies of *Pleurotus pulmonarius* strain P127 yielded on waste paper and straw compost. Amino acids represented by their three-letter codes. ■, straw compost; □, straw compost with 4·5 mM $CdCl_2$; ▤, waste paper compost; ▥, waste paper compost with 4·5 mM $CdCl_2$. Mean values of three replicates.

manganese as well as cadmium (Table 3). When three independent batches of straw and wastepaper were analysed, cadmium was consistently detectable (S. W. Chiu, S. C. Law, K. T. Cheung & M. L. Ching, unpublished result). As shown in Table 3, the straw compost contained more minerals. With the addition of calcium chloride, both the stipe and the pileus showed increased contents of Ca (0·1–1 mg g^{-1}; Fig. 17). Unlike insoluble calcium carbonate (up to 5% (= 625 mM)) which did not have any effect, insoluble calcium hydroxide at 2% (= 338 mM) increased the accumulation of calcium by 3·5-

Table 3. The basal metal contents contained in the compost without exogenous addition of metallic salts

	Content* (μg g^{-1}; D.W.)										
	Pb	Cd	Fe	Al	Zn	Co	Mn	Cu	Ni	Ca	Ni
Straw	0·0648	0·0016	0·0190	90·03	0·2138	0·0119	0·0039	0·2126	0·0217	2·29	0·0217
Waste paper	0·0488	0·0035	0·1082	66·96	0·1271	0·0221	0·0011	0·0310	0·0098	n.d.	0·0098

* Mean values of three replicates. n.d., not determined.



Figs 17–18. Effect of metallic salts on the accumulation of metals by *Pleurotus pulmonarius* strain P127. **Fig. 17.** $CaCl_2$. **Fig. 18.** $MnCl_2$. □, pileus; ○, stipe. Means of five replicates and S.D. Letters indicate the ranking after analysis with ANOVA and Duncan multiple range test. Rank 'a' is the greatest.

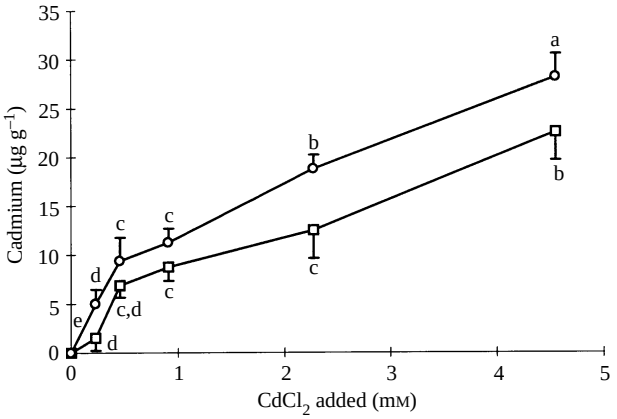
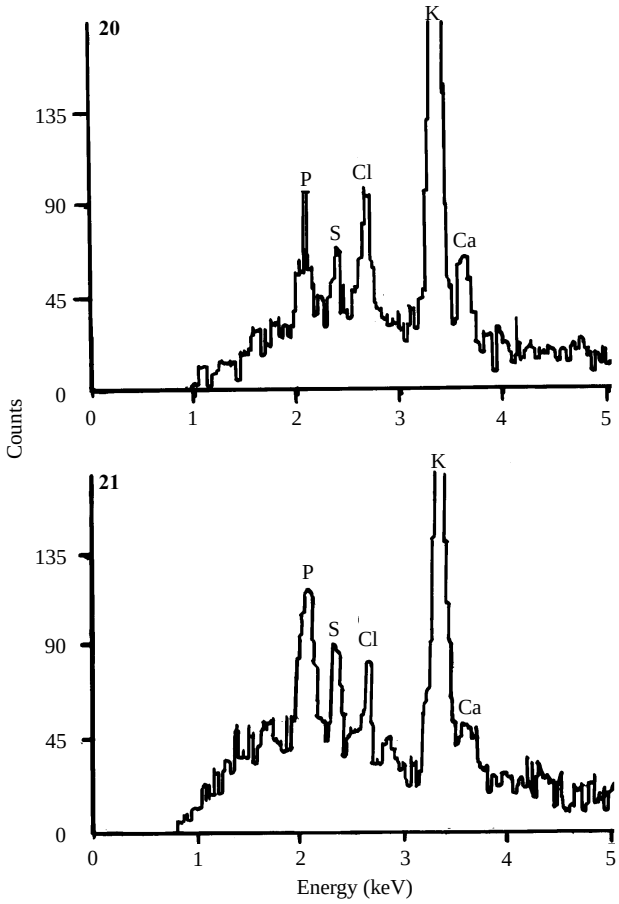


Fig. 19. Effect of $CdCl_2$ on the accumulation of cadmium by *Pleurotus pulmonarius* strain P127. Means of five replicates and S.D. □, straw compost; ○, waste paper compost. Letters indicate the ranking after analysis with ANOVA and Duncan multiple range test. Rank 'a' is the greatest.

fold. When $MnCl_2$ was added, both the pileus and the stipe showed higher manganese contents (Fig. 18) but $MnSO_4$ did not have any significant effect (data not shown). The anion, therefore, affected the amount of metal absorbed by the oyster mushroom. When equivalent amounts of metals were added separately, manganese was accumulated but not to the same extent as the calcium salts (Figs 17, 18). Increasing the concentration of cadmium to 4·5 mM enhanced its accumulation in fruit bodies, and its content reached 28 ± 2 μg g^{-1} D.W. (Fig. 19). In contrast, *P. pulmonarius* did not accumulate aluminium, and this metal had a content of 5 ± 2 μg g^{-1} in both stipe and pileus tissues.



Figs 20–21. Energy dispersive spectra of the fruit body tissues of *Pleurotus pulmonarius* strain P127 as analysed by X-ray spectrometry at 20 keV. No high peaks were observed beyond 5 keV. **Fig. 20.** Stipe. **Fig. 21.** Pileus.

Scanning electron microscopy

SEM revealed that the metallic salts tested did not affect sporulation. Also, no crystalline structure was observed on the surface of the stipe and pileus tissues. The addition of a metallic salt did not affect the surface element composition of the fruit body tissues as revealed by X-ray microanalysis. Typical energy dispersive spectra are shown in Figs 20 and 21.

DISCUSSION

Calcium salts are good to supplement the fruiting substrate

Even without the supplementation of the metallic salts, the straw compost contained the required metals (Table 3) for normal growth and fruiting of the mushroom (Figs 6–11). The element contents, the total amino acid contents between the stipe and the pileus, were different (Table 2; Figs 12–15, 17–18, 20–21) as in other mushrooms (Gruen & Wong, 1981; Latiff *et al*., 1996) with the pileus being more nutritious. This could be because the pileus is the organ for spore production and dispersal.

Among the three metallic salts tested, cadmium toxicity towards *P. pulmonarius* is expressed in terms of increased cadmium content, changed amino acid profile and reduced fruiting yield (Figs 5, 8, 16, 19). Higher concentrations of manganese chloride were required to show these adverse effects (Table 1; Figs 2, 7, 14). Manganese sulphate reduced renewed fruiting (Fig. 4) and retarded growth rate (Table 1) but stimulated fruiting yield (Fig. 9). As manganese is known to be mutagenic, however, the large variation in yield in the replicates might represent some form of mutated mycelial sectors.

The content of manganese in most mushrooms is about one-tenth that of calcium (Tyler, 1980; Mueller *et al*., 1985; Bisara *et al*., 1987). The general effect of increasing the calcium content in the edible mushrooms (Fig. 17) is favoured as calcium is a necessary macronutrient for humans. Daily consumption of 2–3 g (D.W.) of the oyster mushroom fruited in calcium-supplemented compost will meet the recommended dietary allowances for calcium (0·8–1·3 g d^{-1}) by the Food and Nutrition Board, U.S.A. (1989) (cited in Anderson & Garner, 1996). Water-soluble calcium chloride at concentrations below 100 mM caused more rapid colonization of the compost (Table 1) and increased fruit body yield (Fig. 6). Insoluble calcium salts become available only after mobilization by fungal metabolism such as by secreting organic acids (Sayer *et al*., 1995) and represent a slow release form of Ca^{2+} ions. Thus a higher amount of insoluble calcium salts was needed to show a stimulatory effect (Figs 10, 11).

Edible mushrooms are regarded as a food product rich in protein (Chang & Chiu, 1992; Royse, 1995). The crude protein content is conventionally calculated by multiplying the total N content with a conversion factor of 6·25 for mushrooms (Gruen & Wong, 1981; Fujihara *et al*., 1995). Table 2 shows that $CaCl_2$ decreased the total N content which matches with the claim of decreasing the crude protein content by Purkayastha *et al*. (1994). As the total N includes chitin-N and nucleic acid-N (Sosulski & Imafidon, 1990; S. W. Chiu, M. L. Ching & P. N. Lee, unpublished result), the total amino acid content is now accepted as a better indicator of the crude protein content (Fujihara *et al*., 1995; Salo-Vnen & Koivisotoinen, 1996). Fig. 12 shows that the total amino acid content in the pileus, which is the major part for consumption as a food product, increases in the presence of $CaCl_2$. Together with other beneficial effects, application of calcium chloride at or below 100 mM is favourable for cultivation of *P. pulmonarius*. In commercial application, lime ($CaCO_3$) or gypsum ($CaSO_4$) of the agricultural grade is commonly used (Bech & Rasmussen, 1968; Lelley & Janssen, 1993). Thorough mixing with the substrate is always a problem. Using soluble $CaCl_2$ can then provide a solution.

Metal tolerance and toxicity

In fungi, both calcium and manganese, but not cadmium, are needed for growth but the necessary amounts can be different (Harold, 1994; Jennings, 1995). To prevent the damage or immobilization of phosphate caused by excess metallic salts or toxic compounds, a fungus can immobilize the metal ions by simple adsorption or precipitation in cell wall or extracellular matrix, such as formation of calcium oxalate crystals or cadmium sulphide (Whitney & Arnott, 1988; Cunningham & Lundie, 1993). Washing the edible fruit bodies could be a means to remove them. *Pleurotus pulmonarius* may immobilize soluble Mn^{2+} into brown and insoluble manganese (IV) oxide (Gounot, 1994) in buffered medium or may form a brown lignin–polysaccharide complex in straw compost (Shigo, 1974; Gutirez *et al*., 1996). On the other hand, *L. edodes* discriminates against manganese during metal accumulation into fruit bodies by excreting it to the exudate (Leatham & Stahmann, 1989). SEM with the freeze-dried fruit body tissues did not reveal any extracellular crystals, and X-ray microanalysis did not reveal any increase in the corresponding metal content in the wall of the fruit body tissues. Further, the amount of metals desorbed by soaking the fruit body tissues of *P. pulmonarius* in 1 N HCl or in ultrapure water did not differ, and the eluted amounts were trace or undetectable (unpublished result). Most of the metals were, therefore, accumulated intracellularly, as with Mn^{2+} in *S. cerevisiae* (Farcasanu *et al*., 1996). In yeasts, detoxification of cadmium occurs by intracellular chelators; *S. cerevisiae* synthesizes metallothionein which is rich in cysteine [$(Cys\text{-}x\text{-}Cys)_n$] (Macredie *et al*., 1994), and *Schizosaccharomyces pombe* synthesizes phytochelatin which is derived from glutathione [$(\gamma\text{-glutamine-cysteine})_n$-glycine] (Glaser *et al*., 1991; Yukimasa & Norihiro, 1994). Fig. 16 shows that the modulated amino acid profiles of *P. pulmonarius* as a response to the addition of cadmium chloride did not reveal rises in Cys, Glu and Gly. As intracellular soluble ligands were used to bind cadmium in *A. bisporus* (Lind *et al*., 1995), it is possible that a similar system may be present but the specific peptide, if produced, may not be the major product in cells of *P. pulmonarius*.

Tyler (1980) defined the terms bioconcentration and bioexclusion in relation to fungi. Bioconcentration refers to a mushroom with a metal concentration at least 10 times higher than the median of 130 samples collected in the wild. Bioexclusion means a mushroom having a metal concentration

of one-tenth or lower of the median. The median contents were as follows: calcium, 175 μg g^{-1} D.W. (= ppm); manganese, 25 μg g^{-1}; cadmium, 0·6 μg g^{-1} (Tyler, 1980). On Tyler's definition, therefore, *P. pulmonarius* strain P127 bioconcentrates cadmium. Similar accumulation effects of cadmium by *Pleurotus* and *Agaricus* spp. have been reported (Brunnert & Zadražil, 1983; Bisara *et al*., 1987; Purkayastha *et al*., 1994). No obvious symptom was associated with cadmium-contaminated fruit bodies. As FAO/WHO (1972) suggested a provisional tolerable weekly intake of cadmium by adult humans is 0·4–0·5 mg, an intake of 20 g of such heavily contaminated oyster mushroom with normal appearance would exceed this tolerable level and cause a health hazard. Regular monitoring of the metal contents in edible mushrooms in market is, therefore, recommended.

A bioassay system to identify fruiting modulators

The *in vitro* bioassay system using excised stipes is more sensitive and takes less time to reveal the effects of fruiting modulators (e.g. compare Fig. 1 with Fig. 6). This *in vitro* system can, therefore, be used as a fast and convenient bioassay for assessing the effect of substances on fruiting of *P. pulmonarius*. The difference in effective concentration is not only owing to the difference of inoculum (excised stipes in bioassay system and vegetative mycelium in commercial cultivation practice) but due to the complex composition of compost (e.g. lowering the availability of metallic ions by high pH, adsorption of the metallic ions onto straw or its breakdown products, presence of organic chelators; Birch & Bachofen, 1990).

Although mushrooms have been cultivated for a few millennia, our understanding of the fruiting mechanism is poor. Such an *in vitro* bioassay system can be applied as a convenient experimental tool in this aspect.

We thank the British Council for a U.K./Hong Kong joint research scheme grant to S.W.C. and D.M. and a direct grant from the Chinese University of Hong Kong to S.W.C. for partial support of this study. We are also grateful to Miss Pauline Mei-yan Poon for technical assistance and to Professor R. H. Petersen for providing the tester strains for culture identification.

REFERENCES

Anderson, J. B. & Garner, S. C. (1996). *Calcium and Phosphorus in Health and Disease*. CRC Press: Boca Raton, New York, London, Tokyo.

AOAC (1990). *Official Methods of Analysis of the Association of Official Analytical Chemists*, **vols I** and **II**, 15th edn. Association of Official Analytical Chemists, Inc.

Auling, G. (1994). Manganese: function and transport in fungi. In *Metal Ions in Fungi* (ed. G. Winkelmann & D. R. Winge), pp. 215–236. Marcel Dekker: New York, Basel, Hong Kong.

Babich, H. & Stotzky, G. (1981). Manganese toxicity to fungi: influence of pH. *Bulletin of Environmental Contamination and Toxicology* **27**, 474–480.

Bech, K. & Rasmussen, C. R. (1968). Further investigations on organic and inorganic supplementation of mushroom compost. *Mushroom Science* **7**, 329–342.

Beelman, R. B., Miklus, M. B., Mau, J. L., Ajlouni, S. O. & Simons, S. S. (1993). Selected cultural and harvest practices to improve quality and shelf life of *Agaricus* mushrooms. In *Mushroom Biology and Mushroom Products* (ed. S.-T. Chang, J. A. Buswell & S. W. Chiu), pp. 177–184. The Chinese University Press: Hong Kong.

Birch, L. & Bachofen, R. (1990). Complexing agents from micro-organisms. *Experientia* **46**, 827–834.

Bisara, R., Madan, M. & Bisaria, V. S. (1987). Mineral content of the mushroom *Pleurotus sajor-caju* cultivated on different agro-residues. *Mushroom Journal for the Tropics* **7**, 53–60.

Bresinsky, A., Fischer, M., Meixner, B. & Paulus, W. (1987). Speciation in *Pleurotus*. *Mycologia* **79**, 234–245.

Brunnert, H. & Zadražil, F. (1983). The translocation of mercury and cadmium into the fruiting bodies of six higher fungi. A comparative study on species specificity in five lignocellulolytic fungi and the cultivated mushroom *Agaricus bisporus*. *European Journal of Applied Microbiology and Biotechnology* **17**, 358–364.

Camarero, S., Bockle, B., Martinez, M. J. & Martinez, A. T. (1996). Manganese-mediated lignin degradation by *Pleurotus pulmonarius*. *Applied and Environmental Microbiology* **62**, 1000–1072.

Chang, S. T. & Chiu, S. W. (1992). Mushroom production – an economic measure in maintenance of food security. In *Microbial Technology: Economic and Social Aspects* (ed. E. J. DaSilva, C. Ratledge and A. Sasson), pp. 110–141. Cambridge University Press: Cambridge, U.K.

Chiu, S. W. & Moore, D. (1988). Evidence for developmental commitment in the differentiating fruit body of *Coprinus cinereus*. *Transactions of the British Mycological Society* **90**, 247–253.

Chiu, S. W. & Moore, D. (1990). A mechanism for gill pattern formation in *Coprinus cinereus*. *Mycological Research* **94**, 320–326.

Chiu, S. W. & Poon, Y. K. (1993). Submerged production of *Monascus* pigments. *Mycologia* **85**, 214–218.

Chiu, S. W. & To, S. W. (1993). Endogenous glycogen is not a trigger for fruiting in *Pleurotus sajor-caju*. *Mycological Research* **97**, 363–366.

Cunningham, D. P. & Lundie, L. L. Jr (1993). Precipitation of cadmium by *Clostridium thermoaceticum*. *Applied and Environmental Microbiology* **59**, 7–14.

FAO/WHO (1972). *Evaluation of Certain Food Additives and Contaminants: Mercury, Lead and Cadmium*. 16th Report of Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series no. 505.

Farcasanu, I. C., Ohta, N. & Miyakawa, T. (1996). The fate of Mn^{2+} ions inside *Saccharomyces cerevisiae* cells seen by electron paramagnetic resonance. *Bioscience, Biotechnology and Biochemistry* **60**, 468–471.

Frank, V., Tamova, G. & Farkas, V. (1994). Characterization of a white mutant of *Trichoderma viride* obtained by cadmium treatment. *Microbiological Research* **149**, 61–64.

Fujihara, S., Kasuga, A., Aoyagi, Y. & Sugahara, T. (1995). Nitrogen-to-protein conversion factors for some common edible mushrooms. *Journal of Food Science* **60**, 1045–1047.

Gabriel, J., Mokrejš, M., Bý, J. & Rychlovský, P. (1994). Accumulation of heavy metals by some wood-rotting fungi. *Folia Microbiologica* **39**, 115–118.

Gehrke, C. W., Wall, L. L. Sr, Absheer, J. S., Kaiser, F. E. & Zumwalt, R. W. (1985). Sample preparation for chromatography of amino acids: acid hydrolysis of proteins. *Journal of the Association of Official Analytical Chemists* **68**, 811–821.

Glaser, H., Coblenz, A., Kurczek, R., Ruttke, I., Ebert-Jung, A. & Wolf, K. (1991). Glutathione metabolism and heavy metal detoxification in *Schizosaccharomyces pombe*. *Current Genetics* **19**, 207–213.

Good, N. E., Winget, G. D., Winter, W., Connolly, T. N., Izawa, S. & Singh, R. M. M. (1966). Hydrogen ion buffers for biological research. *Biochemistry* **5**, 467–477.

Gounot, A.-M. (1994). Microbial oxidation and reduction of manganese: consequences in ground water and applications. *FEMS Microbiology Reviews* **14**, 339–350.

Gruen, H. E. & Wong, W. M. (1981). Distribution of cellular amino acids, protein, and total organic nitrogen during fruitbody development in *Flammulina velutipes*. II. Growth on potato–glucose solution. *Canadian Journal of Botany* **60**, 1342–1351.

Gutirez, A., Bocchini, P., Galletti, G. C. & Martinez, A. T. (1996). Analysis of lignin–polysaccharide complexes formed during grass lignin degradation by cultures of *Pleurotus* species. *Applied and Environmental Microbiology* **62**, 1928–1934.

Haldimann, M., Bajo, C., Haller, T., Venner, T. & Zimmerli, B. (1995). [Occurrence of arsenic, lead, cadmium, mercury and selenium in cultivated

mushrooms.] *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* **86(5)**, 463–484. [In German with English summary.]

Harold, F. M. (1994). Ionic and electrical dimensions of hyphal growth. In *The Mycota*, **vol. 1**. *Growth, Differentiation and Sexuality* (ed. J. G. H. Wessels & F. Meinhardt), pp. 89–109. Springer-Verlag: Berlin.

Hayes, W. A. (1972). Nutritional factors in relation to mushroom production. *Mushroom Science* **8**, 662–674.

Hughes, M. N. & Poole, R. K. (1989). *Metals and Micro-organisms*. Chapman & Hall: London.

Iracabal, B., Zervakis, G. & Labare, J. (1995). Molecular systematics of the genus *Pleurotus*: analysis of restriction length polymorphisms in ribosomal DNA. *Microbiology* **141**, 1479–1490.

Jain, S. K., Gujral, G. S., Vasudevan, P. & Jha, N. K. (1989). Uptake of heavy metals by *Azolla pinnata* and their translocation into the fruitbodies of *Pleurotus sajor-caju*. *Journal of Fermentation and Bioengineering* **68**, 64–67.

Jennings, D. H. (1995). *The Physiology of Fungal Nutrition*. Cambridge University Press: Cambridge, U.K.

Keen, C. L., Zidenberg-Cher, S. & Lonnerdal, B. (1994). Nutritional and toxicological aspects of manganese intake: an overview. In *Risk Assessment of Essential Elements* (ed. W. Mertz, C. O. Abernathy & S. S. Olin), pp. 221–236. ILSI Press: Washington, D.C.

Kerem, Z. & Hadar, Y. (1993). Effects of manganese on lignin degradation by *Pleurotus ostreatus* during solid-state fermentation. *Applied and Environmental Microbiology* **59**, 4115–4120.

Latiff, L. A., Daran, A. B. M. & Mohamed, A. B. (1996). Relative distribution of minerals in the pileus and stalk of some selected edible mushrooms. *Food Chemistry* **56**, 115–121.

Leatham, G. F. & Stahmann, M. A. (1989). The effects of common nutritionally-important cations on the growth and development of the cultivated mushroom *Lentinula edodes*. *Mushroom Science* **12**, 253–265.

Lelley, J. I. & Janssen, A. (1993). Productivity improvement of oyster mushroom substrate with a controlled release nutrient. *Mushroom News* **41**, 6–13.

Lepsova, A. & Mejstrik, V. (1989). Trace elements in fruit bodies of fungi under different pollution stress. *Agriculture, Ecosystems and Environment* **28**, 305–312.

Levanon, D. (1993). Mushroom cultivation: environmental problem to environmental benefit. In *Mushroom Biology and Mushroom Products* (ed. S.-T. Chang, J. A. Buswell & S. W. Chiu), pp. 331–338. The Chinese University Press: Hong Kong.

Lind, Y., Glynn, A. W., Engman, J. & Jorhem, L. (1995). Bioavailability of cadmium from crab hepatopancreas and mushroom in relation to inorganic cadmium: a 9-week feeding study in mice. *Food and Chemical Toxicology* **33**, 667–673.

Macredie, G. I., Sewell, A. K. & Winge, D. R. (1994). Metal ion resistance and the role of methallothionein in yeast. In *Metal Ions in Fungi* (ed. G. Winkelmann & D. R. Winge), pp. 279–309. Marcel Dekker: New York.

Matsumoto, T. & Fukumasa-Nakai, Y. (1994). Isolation and genetic analysis of a chloramphenicol-resistant mutant of *Pleurotus ostreatus*. *Bioscience, Biotechnology and Biochemistry* **58**, 434–435.

McDougall, D. N. & Blanchette, R. A. (1996). Metal ion adsorption by pseudosclerotial plates of *Phellinus weirii*. *Mycologia* **88**, 98–103.

Miklus, M. B. & Beelman, R. B. (1996). $CaCl_2$ treated irrigation water applied to mushroom crops (*Agaricus bisporus*) increases calcium concentration and improves postharvest quality and shelf life. *Mycologia* **88**, 403–409.

(*Accepted 8 September 1997*)

Mueller, J. C., Gawley, J. R., Lanz, H. & Hayes, W. A. (1985). Mineral and heavy metal content of *Pleurotus sajor-caju* grown on cellulosic residues from a bleached kraft pulp mill. *Mushroom Newsletter for the Tropics* **5**, 9–16.

Petersen, R. H. & Hughes, K. W. (1993). Intercontinental interbreeding collections of *Pleurotus pulmonarius*, with notes on other species. *Sydowia* **45**, 139–152.

Piscator, M. (1988). Some views on metabolism, toxicity and carcinogenicity of cadmium. In *Environmental Toxin Series 2, Cadmium* (ed. M. Stoeppler & M. Piscator), pp. 3–12. Springer-Verlag: Berlin, Heidelberg.

Purkayastha, R. P., Mitra, A. K. & Bhattacharyya, B. (1994). Uptake and toxicological effects of some heavy metals on *Pleurotus sajor-caju* (Fr.) Singer. *Ecotoxicology and Environmental Safety* **27**, 7–13.

Ragunathan, R., Gurusamy, R., Palaniswamy, M. & Swaminathan, K. (1996). Cultivation of *Pleurotus* spp. on various agro-residues. *Food Chemistry* **55**, 139–144.

Royse, D. J. (1995). Specialty mushrooms. *Mushroom News* **43**, 4–21.

Salo-Vnen, P. P. & Koivisotoinen, P. E. (1996). Determination of protein in foods: comparisons of net protein crude protein ($N \times 6{\cdot}5$) values. *Food Chemistry* **57**, 27–31.

Sanglimsuwan, S., Yoshid, N., Morinaga, T. & Mrooka, Y. (1993). Resistance to and uptake of heavy metals in mushrooms. *Journal of Fermentation and Bioengineering* **75**, 112–114.

Sayer, J. A., Ragett, S. L. R. & Gadd, G. M. (1995). Solubilization of insoluble metal compounds by soil fungi: development of a screening method for solubilizing ability and metal tolerance. *Mycological Research* **99**, 987–993.

Shigo, A. L. (1974). Effects of manganese, calcium, zinc and iron on growth and pigmentation of *Trichocladium canadense*, *Phialophora melinii*, *Hypoxylon rubiginosum*, *Daldinia concentrica* and *Cytospora decipiens*. *Mycologia* **66**, 339–341.

Sosulski, F. W. & Imafidon, G. I. (1990). Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. *Journal of Agricultural and Food Chemistry* **38**, 1351–1356.

Tathvonen, R. (1996). Contents of lead and cadmium in foods and diets. *Food Review International* **12**, 1–70.

Tsang, L. J., Reid, I. D. & Coxworth, E. C. (1987). Delignification of wheat straw by *Pleurotus* spp. under mushroom growing conditions. *Applied and Environmental Microbiology* **53**, 1304–1306.

Tyler, G. (1980). Metals in sporophores of basidiomycetes. *Transactions of the British Mycological Society* **74**, 41–49.

Vetter, J. (1994). Data on arsenic and cadmium contents of some common mushrooms. *Toxicon* **32**, 11–15.

Waalkes, M. P. & Oberdorster, G. (1990). Cadmium carcinogenesis. In *Biological Effects of Heavy Metals*, **vol. II**. *Metal Carcinogenesis* (ed. E. C. Foulkes), pp. 130–157. CRC Press: Boca Raton.

Whitney, K. D. & Arnott, H. J. (1988). The effect of calcium on mycelial growth and calcium oxalate crystal formation in *Gilbertella persicaria* (Mucorales). *Mycologia* **80**, 707–715.

Yukimasa, H. & Norihiro, M. (1994). Cadystin (phytochelatin) in fungi. In *Metal Ions in Fungi* (ed. G. Winkelmann & D. R. Winge), pp. 311–337. Marcel Dekker: New York.

Zervakis, G. & Balis, C. (1996). A pluralistic approach in the study of *Pleurotus* species with emphasis on compatibility and physiology of the European morphotaxa. *Mycological Research* **100**, 717–731.