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| **Project Title** | Mushrooms: Reducing cadmium levels in horse mushrooms |
| **Project number:** | M 45 |
| **Project leader:** | Ralph Noble, Warwick HRI |
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| **Location of project:** | Warwick HRI |
| **Project coordinator:** | Mark Komatsu, Oakfield Farm Products |
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**Signed on behalf of: Warwick HRI**

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## Grower Summary

### Headline

- Availability of cadmium in compost to horse mushrooms depends not only on the cadmium content of the compost, but also on its pH values at the start and end of cultivation (low pH increases the availability of cadmium).
- Poultry manure is the main source of cadmium in compost and sugar beet lime is the main source of cadmium in casing.
- Horse mushrooms can be produced with cadmium levels below the new EU limit (1 mg $kg^{-1}$ fresh weight).

### Background

The tendency for horse mushrooms (*Agaricus arvensis*) to accumulate heavy metals is well established. The cadmium content of horse mushrooms resulted in them exceeding the EU regulation on contaminants in foodstuffs (466/2001 and 1881/2006) and being withdrawn from sale. The EU regulation set a cadmium limit for cultivated fungi of 0.2 mg/kg wet (fresh) weight. This limit also affected other exotic species that may have a tendency to accumulate heavy metals.

However, an amendment to this limit was agreed during 2008. The current limit for cadmium of 0.2 mg/kg wet weight will only apply to *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*; the limit for all other species of edible fungi has been increased to 1 mg/kg wet weight. The effect of the cadmium content of cultivation substrates on the cadmium content of horse mushrooms had not previously been investigated.

### Objectives and expected deliverables

01 Determine the cadmium contents of straw, nitrogen and gypsum sources for compost.

02 Determine the cadmium content of casing ingredients

03 Using information from (01) and (02), prepare composts and casings and compare the yield and cadmium content of cultivated horse mushrooms with those grown in standard straw/poultry manure compost and peat/lime casings.

04 Disseminate results to industry; explore commercial feasibility and uptake of results.

## Summary of the project

### Method

Compost ingredients and proportions used in different treatments are shown in Table 1. Composts ‘Normal A’ and ‘Normal B’ were prepared from wheat straw, poultry manure and gypsum. Composts ‘Low C’ and ‘Low D’ were prepared from wheat straw, ammonium sulphate, urea and gypsum; ‘Low C’ also contained vegetable waste (a mixture of 40% brassica stems and leaves, 40% potatoes and root vegetables and 20% tomato haulms and fruit). A lower rate of gypsum (calcium sulphate) was used in the composts containing ammonium sulphate (Table 1).

**Table 1.** Percentage by weight of ingredients used in composts, excluding added water.

| Treatment name | Straw | | Other ingredient | |
|---|---|---|---|---|
| | Type | w/w | Type | w/w |
| A Normal | Wheat A | 60.6 | Poultry manure A | 35.4 |
| | | | Gypsum | 4.0 |
| B Normal | Wheat B | 60.6 | Poultry manure B | 35.4 |
| | | | Gypsum | 4.0 |
| C Low | Wheat | 47.3 | Vegetable waste | 47.3 |
| | | | Ammonium sulphate | 2.9 |
| | | | Urea | 0.7 |
| | | | Gypsum | 1.8 |
| D Low | Wheat | 90.1 | Ammonium sulphate | 4.5 |
| | | | Urea | 1.8 |
| | | | Gypsum | 3.6 |

Peat and lime sources were analysed for cadmium content. Based on these results, a ‘Normal Casing A’ based on dry milled peat and sugar beet lime (15% v/v) and a low cadmium casing (Low Casing B) based on wet-dug peat and chalk (15% w/w) were prepared.

The following combination treatments of composts and casings were prepared to determine the relative contributions of each substrate to the cadmium content of the mushrooms:

| Treatment | Compost | Casing |
|---|---|---|
| 1 | A Normal cadmium | A Normal cadmium |

| | | |
|---|---|---|
| 2 | A Normal cadmium | B Low cadmium |
| 3 | B Normal cadmium | A Normal cadmium |
| 4 | B Normal cadmium | B Low cadmium |
| 5 | C Low cadmium | A Normal cadmium |
| 6 | C Low cadmium | B Low cadmium |
| 7 | D Low cadmium | A Normal cadmium |
| 8 | D Low cadmium | B Low cadmium |

**Main conclusions**

1. Poultry manure was the main source of cadmium in compost and sugar beet lime was the main source of cadmium in casing; composts and casing with low cadmium contents were prepared by using poultry manure and lime with a low cadmium content (0.24 mg $kg^{-1}$ dry weight) and/or by reducing or avoiding these raw materials.
2. Availability of cadmium in compost to horse mushrooms depended not only on the cadmium content of the compost, but also on its pH values at the start and end of cultivation; composts with a low cadmium content but also a low pH still produced horse mushrooms with a high cadmium content in the first flush.
3. The cadmium content of the casing layer had a smaller effect on the cadmium content of horse mushrooms than the cadmium content of the compost; reducing the cadmium content of the casing by replacing sugar beet lime with chalk resulted in a small reduction in the cadmium content of horse mushrooms.
4. The cadmium contents of all the first and second flush horse mushrooms in these experiments were below the new EU limit (1 mg $kg^{-1}$ fresh weight) irrespective of the compost and casing treatments.
5. To achieve the previous EU limit for cadmium levels in horse mushrooms (0.2 mg $kg^{-1}$ fresh weight), the critical cadmium content in the compost and casing was about 0.3 mg $kg^{-1}$ dry weight; this maximum cadmium level needed to be combined with compost and casing pH values that did not fall below about 6.85 during cultivation.
6. The best yields of horse mushrooms were obtained using a compost containing vegetable waste and casing prepared from wet peat and chalk.

**Financial and environmental benefits**

This project identified practical commercial methods for for reducing the cadmium content of horse mushrooms to below the EU regulation limit. Using these findings UK growers can therefore produce the crop once more. This presents an opportunity for additional income.

**Action points for composters and growers**

- The effect of increasing the pH of the compost and casing on the cadmium content and yield of horse mushrooms and other species should be investigated in commercial crops. Increasing the compost pH could be achieved by adding urea, chalk ($CaCO_3$) and/or hydrated lime (CaOH) during or after the Phase I composting process, or by adding the latter two materials after Phase II compost is made.
- Raw materials used for preparing horse mushroom substrates, particularly poultry manure and lime sources, should be routinely tested for cadmium content. The results of this work indicate that this should not exceed 0.3 mg $kg^{-1}$ dry weight.
- If possible, poultry manure in compost should be partly substituted by vegetable waste and urea (but not ammonium sulphate, which reduces compost pH).
- Chalk should replace sugar beet lime in casing used for horse mushroom cultivation.

## Science Section

### Introduction

The strains of horse mushroom (*Agaricus arvensis*) used in commercial culture (Sylvan R20, Mycelia M 7400) were developed by Fritsche (1979). An HDC project (M4b) showed that yields of about 170 kg /tonne compost could be obtained in three flushes (Noble, 1995). However, the cadmium content of horse mushrooms resulted in crops exceeding the EU regulation on contaminants in foodstuffs (466/2001 and 1881/2006) and being withdrawn from sale (Anon., 2001, 2006). The EU regulation set a cadmium limit for cultivated fungi of 0.2 mg/kg wet (fresh weight) weight. This limit also affected other exotic species that may have a tendency to accumulate heavy metals. However, an amendment to this limit has recently been agreed. The current limit for cadmium of 0.2 mg/kg wet weight will only apply to *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*; the limit for all other species of edible fungi has been increased to 1 mg/kg wet weight.

The tendency for *Agaricus* species in the *Flavescentes* Section, which includes *A. arvensis* and *A. blazei*, to accumulate heavy metals is well established. Movitz (1980) found that the cadmium content of 58 wild samples of *A. arvensis* ranged from 0.1 to 22.5 mg/kg. The highest cadmium concentrations were in the top of the mushroom and the lowest were in the base. Woggon & Bickerich (1978) obtained a range in cadmium contents of 0.06 to 2.58 mg/kg for 23 samples of wild *A. arvensis*, assuming a dry matter content of 8%. Kruse & Lommel (1979) obtained an average cadmium content of 5 mg/kg in 42 samples of wild *A. arvensis*. A MAFF survey found cadmium contents for wild *A. arvensis* of 0.05 to 5.30 mg/kg (Anon, 2000). Unpublished data from the Campden and Chorleywood Food Research Association (CCFRA) gives cadmium contents of 0.31 to 0.64 mg/kg for cultivated samples of *A. arvensis*. The effect of the cadmium content of substrates on the cadmium content of cultivated horse mushrooms has not been investigated.

*Agaricus* species in the *Rubescentes* Section, which includes *Agaricus bisporus*, have a much lesser tendency to accumulate heavy metals than *Agaricus* species in the *Flavescentes* Section. The cadmium level in a wild *A. bisporus* was 0.06 mg/kg (Movitz, 1980) and the cadmium levels in cultivated *A. bisporus* samples were also generally less than 0.12 mg/kg (Thomas et al, 1972; Fleckenstein & Grabbe, 1981; Amsing, 1983; Stelte et al, 1983). Fleckenstein & Grabbe (1981) found that *A. bisporus* grown in a compost containing 50% municipal waste and 2.20 mg/kg cadmium had a significantly higher cadmium content (0.20 mg/kg) than mushrooms grown in a horse manure compost containing 0.84 mg/kg cadmium (0.10 mg Cd/kg mushrooms). Stelte et al (1983) found a relationship between the cadmium content of substrates and the resulting cadmium content of mushrooms. However, Amsing (1983) found no such relationship, although he did find a close relationship between the mercury contents of composts and the mushrooms produced. Mushroom compost samples analysed by Chipping Campden and Chorleywood Research Association (CCFRA) had cadmium contents of 0.05 and 0.06 mg/kg. Fritsche (1989) recommended the use of phosphate fertiliser to reduce the pH of the compost used for producing horse mushrooms. However, the cadmium content of phosphatic fertilisers has resulted in increases in the cadmium content of vegetables grown on treated soils (Thomas et al, 1972) and this may also be a cause of some straw batches used in making compost having higher cadmium contents.

Noble & Dobrovin-Pennington (2005) found that sugar beet lime used in casing had a cadmium content of 0.6 mg/kg. However, the effect of the cadmium content of the casing layer on the cadmium content of mushrooms has not been investigated.

The overall aim of this project is to reduce the cadmium content of cultivated horse mushrooms to below 0.2 mg/kg while maintaining a high yield (170 kg/tonne in three flushes). Specific objectives of the work are:

- To identify suitable low cadmium compost and casing ingredients that can be used for cultivating horse mushrooms.
- To determine the relative importance of the cadmium content of the compost and of the casing as sources of cadmium to the cultivated horse mushroom.

**Commercial Objectives**

01 Determine the cadmium contents of straw, nitrogen and gypsum sources for compost.

02 Determine the cadmium content of casing ingredients

03 Using information from (01) and (02), prepare composts and casings and compare the yield and cadmium content of cultivated horse mushrooms with those grown in standard straw/poultry manure compost and peat/lime casings.

04 Disseminate results to industry; explore commercial feasibility and uptake of results.

**Materials and methods**

*Compost production*

Carbon and nitrogen sources for compost production were selected from HDC Project M 43 (Noble et al., 2008). The materials were analysed for pH and N, ammonium N, ash and moisture contents.

Compost ingredients and proportions used in different treatments are shown in Table 1. Composts 'Normal A' and 'Normal B' were prepared from wheat straw, poultry manure and gypsum. Composts 'Low C' and 'Low D' were prepared from wheat straw, ammonium sulphate, urea and gypsum; 'Low C' also contained vegetable waste (a mixture of 40% brassica stems and leaves, 40% potatoes and root vegetables and 20% tomato haulms and fruit). A lower rate of gypsum (calcium sulphate) was used in the composts containing ammonium sulphate (Table 1).

**Table 1.** Percentage by weight of ingredients used in composts, excluding added water.

| Treatment name | Straw | | Other ingredient | |
|---|---|---|---|---|
| | Type | w/w | Type | w/w |
| A Normal | Wheat A | 60.6 | Poultry manure A | 35.4 |
| | | | Gypsum | 4.0 |
| B Normal | Wheat B | 60.6 | Poultry manure B | 35.4 |
| | | | Gypsum | 4.0 |
| C Low | Wheat | 47.3 | Vegetable waste | 47.3 |
| | | | Ammonium sulphate | 2.9 |
| | | | Urea | 0.7 |
| | | | Gypsum | 1.8 |
| D Low | Wheat | 90.1 | Ammonium sulphate | 4.5 |
| | | | Urea | 1.8 |
| | | | Gypsum | 3.6 |

Composts (about 1.2 tonnes of raw materials, excluding water, for each treatment) were prepared in turned windrows during an 18 –day period as described in project report M 43. Due to the slow degradation of the compost prepared only with inorganic nitrogen sources (D Low), theses materials were composted for a further period of 20 days. At the end of Phase I, all the composts had a moisture content of 77 (± 1) % w/w and total nitrogen content of 2.01 (± 0.10) % of dry matter. The ammonium N content was 0.37 (±0.10) % of dry matter, except for compost D Low (0.98 % of dry matter).

Composts were pasteurised in bulk tunnels as described in project report M 43. The materials were then analysed as described for raw ingredients.

*Preparation of casing materials*

Peat and lime sources were analysed for cadmium content. Based on these results, a 'Normal Casing A' based on dry milled peat and sugar beet lime (15% v/v) and a low cadmium casing (Low Casing B) based on wet-dug peat and chalk (15% w/w) were prepared.

*Cropping procedure and experimental layout*

The following combination treatments of composts and casings were prepared to determine the relative contributions of each substrate to the cadmium content of the mushrooms:

| Treatment | Compost | Casing |
|---|---|---|
| 1 | A Normal cadmium | A Normal cadmium |
| 2 | A Normal cadmium | B Low cadmium |
| 3 | B Normal cadmium | A Normal cadmium |
| 4 | B Normal cadmium | B Low cadmium |
| 5 | C Low cadmium | A Normal cadmium |
| 6 | C Low cadmium | B Low cadmium |
| 7 | D Low cadmium | A Normal cadmium |
| 8 | D Low cadmium | B Low cadmium |

Composts were spawned at 2% w/w with rye grain spawn of the strain R20 into 10-L plastic pots, each containing 3 kg of spawned compost. The pots were incubated at 25°C for 15 days and then cased with 700 g of either of the two casings. Two pots of each compost and casing treatment combination were prepared (16 pots in total). The pots were then incubated in polythene bags for a further 7-9 days until mycelium was visible at the surface of the casing. The pots were then moved to a cropping room (18°C, 87% RH, 1000 ppm $CO_2$) with lighting (minimum 150 lux). The number and yield of mushrooms of three flushes, and the cadmium content of mushroom in the first two flushes were recorded. At the end of the cropping procedure, samples of compost and casing from each treatment were analysed for pH.

*Cadmium analysis*

Dried samples of compost and casing ingredients, prepared composts and casings and mushroom samples were analysed for cadmium content by CCFRA Technology Ltd, Chipping Campden using the following procedure. Samples were homogenised to produce representative samples, and then digested in nitric acid using a microwave/pressure digestion system. The digested sample was then diluted to a known volume and analysed by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). All samples were analysed in duplicate to obtain a mean result. All batches of samples were digested and analysed alongside a certified reference material and reagent blank.

## Results

*Analysis of composts and casings*

Straw had a low cadmium content relative to poultry manure, with vegetable waste intermediate on a dry weight basis (Table 2). The cadmium content of the inorganic substrate ingredients (gypsum, ammonium sulphate, urea) and spawn was either very low or not detectable (Table 2).

Poultry manure compost A had a slightly higher cadmium content than poultry manure compost B. This was expected in consideration of the higher cadmium content of the poultry manure used in compost A (Table 2). The cadmium contents, both on a fresh and dry weight basis, of the composts prepared with poultry manure (composts A and B) were higher than those of composts prepared with vegetable wastes and/or inorganic nitrogen sources (C and D) (Table 3). The latter composts had higher moisture and ammonium nitrogen contents but lower pH values and electrical conductivities than the poultry manure-based composts (Table 3).

The milled peat used for casing had a higher cadmium content than the wet peat, and the sugar beet lime (SBL) had a higher cadmium content than the chalk (Table 4). These values are reflected in the mixed casings with the milled peat + SBL casing (A) having a higher cadmium content than the wet peat + chalk casing (B)(Table 5). The pH values of the two casings were similar but the milled peat + SBL casing (A) had a lower moisture content and higher electrical conductivity at application than casing (B) (Table 5).

During cropping, the pH of the composts decreased whereas the pH of the casing increased slightly (Table 6). At the end of cropping, composts prepared with poultry manure (A and B) again had a higher pH than those prepared without (C and D), with compost A having a higher final pH than compost B.

**Table 2.** Analysis of straw types and other compost ingredients.

 

| Ingredient | DM % | % of DM | | | pH | Cd mg kg$^{-1}$ | |
|---|---|---|---|---|---|---|---|
| | | N | $NH_4^+$ | Ash | | FW | DW |
| Wheat straw A 2006 | 12.0 | 0.57 | 0.015 | 7.0 | 6.91 | 0.03 | 0.04 |
| Wheat straw B 2007 | 19.7 | 0.35 | <0.010 | 9.2 | 8.35 | 0.04 | 0.05 |
| Barley straw | 13.1 | 0.70 | 0.034 | 6.0 | 7.21 | 0.06 | 0.07 |
| Rye straw | 11.9 | 0.43 | 0.027 | 7.3 | 6.88 | 0.03 | 0.04 |
| Poultry manure (A) | 36.4 | 5.05 | 0.678 | 12.8 | 9.93 | 0.28 | 0.44 |
| Poultry manure (B) | 41.6 | 5.16 | 1.288 | 19.6 | 8.08 | 0.14 | 0.24 |
| Vegetable waste | 23.9 | 0.86 | 0.123 | 64.7 | 4.43 | 0.05 | 0.20 |
| Gypsum | 99.6 | - | - | - | 8.20 | 0.02 | 0.02 |
| Ammonium sulphate | 99.2 | 21.20 | 27.270 | - | 4.66 | 0.02 | 0.02 |
| Urea | 99.5 | 46.70 | - | - | 8.59 | <0.01 | <0.01 |
| Spawn | 47.6 | 2.9 | 0.040 | 7.0 | 5.66 | 0.02 | 0.04 |

**Table 3.** Analysis of pasteurised (Phase II) composts used in the experiments. Each value is the mean of two replicate samples.

| Treatment | moisture | % of DM | | | pH | EC | Cd mg kg$^{-1}$ | |
|---|---|---|---|---|---|---|---|---|
| | % | N | $NH_4^+$ | Ash | | mS cm$^{-1}$ | FW | DW |
| A Normal | 71.1 | 3.07 | 0.15 | 24.9 | 7.66 | 4.58 | 0.09 | 0.32 |
| B Normal | 67.1 | 3.16 | 0.02 | 27.1 | 7.41 | 5.59 | 0.09 | 0.27 |
| C Low | 73.6 | 2.53 | 0.34 | 24.2 | 6.87 | 3.23 | 0.04 | 0.16 |
| D Low | 73.8 | 2.99 | 1.14 | 25.3 | 6.24 | 2.23 | 0.04 | 0.15 |

**Table 4.** Analysis of casing ingredients.

| Treatment | DM % | pH | EC | Cd mg kg$^{-1}$ | |
|---|---|---|---|---|---|
| | | | mS cm$^{-1}$ | FW | DW |
| Milled peat | 31.5 | 3.40 | 0.25 | 0.04 | 0.13 |
| Wet-dug peat | 12.7 | 4.92 | 0.07 | <0.01 | 0.04 |
| Chalk | 99.8 | 8.93 | 0.34 | 0.26 | 0.26 |
| Sugar beet lime | 68.4 | 8.81 | 0.93 | 0.43 | 0.59 |

**Table 5.** Analysis of casings used in the experiments. Each value is the mean of two replicate samples.

| Casing | Moisture | pH | EC | Cd mg kg$^{-1}$ | |
|---|---|---|---|---|---|
| treatment | % | | mS cm$^{-1}$ | FW | DW |
| A Normal | 73.0 | 7.16 | 597 | 0.09 | 0.32 |
| B Low | 77.5 | 7.03 | 330 | 0.03 | 0.13 |

**Table 6.** pH values of composts and casing at the end of the cropping experiments. Each value is the mean of two replicate samples.

| Substrate | pH |
|---|---|
| Compost A | 6.60 |
| Compost B | 6.85 |
| Compost C | 6.24 |
| Compost D | 5.53 |
| Casing A | 7.35 |
| Casing B | 7.14 |

*Yield of horse mushrooms*

The wet peat + chalk casing (B Low) produced a higher mushroom yield than the milled peat + SBL casing (A Normal) (Table 7). Differences in yield between compost treatments, particularly using the casing B, were fairly small. Compost prepared with vegetable waste and inorganic nitrogen (C Low) produced a higher yield than compost prepared only with inorganic nitrogen (D Low) or compost A prepared with poultry manure (Table 7). Yields from the pot experiments were generally lower than those that can be achieved in larger-scale culture in trays or bags (about 170 kg t$^{-1}$ substrate in three flushes) (Noble, 1995).

**Table 7.** Yield of horse mushrooms in three flushes (g $kg^{-1}$ spawned compost). Each value is the mean of two replicate crops.

| Compost | Casing | | Mean |
|---|---|---|---|
| | A Normal | B Low | (LSD = 21) |
| A Normal | 85 | 121 | 103 |
| B Normal | 115 | 120 | 118 |
| C Low | 131 | 145 | 138 |
| D Low | 98 | 123 | 110 |
| Mean (LSD = 15) | 107 | 127 | |

*Dry matter and cadmium content of horse mushrooms*

The average dry matter content of the mushrooms was 10.6% and was not significantly different between the first and second flushes. Mushrooms grown on Compost B had a slightly higher dry matter content (11.6%) compared with mushrooms grown on Compost C (10.3%). Mushrooms grown with Casing A had a slightly higher dry matter content (11.6%) than mushrooms grown with Casing B (10.5%).

Mushrooms grown on Composts A and B containing poultry manure had a higher cadmium content (fresh or dry weight basis) in the second flush than in the first flush (Tables 8 and 9). However, mushrooms grown on Composts C and D prepared from vegetable waste and/or inorganic nitrogen had a higher cadmium content in the first flush than in the second flush. The highest cadmium contents were recorded in the first flush of mushrooms grown on Composts C and D. Overall, mushrooms grown on Compost B had a lower cadmium content than mushrooms grown on Composts A, C and D. Compost B was prepared using poultry manure with a lower cadmium content than that used for preparing Compost A.

Mushrooms grown on Casing A containing milled peat and sugar beet lime had a slightly higher cadmium content on a fresh weight basis than mushrooms grown on Casing B containing wet peat and chalk (Table 8). However, the difference in cadmium content between mushrooms grown on the two casings was not significant on a dry weight basis (Table 9).

**Table 8.** Cadmium content of horse mushrooms (mg $kg^{-1}$ fresh weight).

Each value is the mean of two replicate crops.

| Compost | Casing | | | | Mean |
|---|---|---|---|---|---|
| | A Normal | | B Low | | (LSD = 0.04) |
| | 1st Flush | 2nd Flush | 1st Flush | 2nd Flush | |
| A Normal | 0.12 | 0.40 | 0.32 | 0.39 | 0.31 |
| B Normal | 0.09 | 0.25 | 0.06 | 0.19 | 0.14 |
| C Low | 0.55 | 0.11 | 0.36 | 0.12 | 0.28 |
| D Low | 0.73 | 0.30 | 0.37 | 0.34 | 0.43 |
| Mean (LSD = 0.03) | 0.32 | | 0.27 | | |

**Table 9.** Cadmium content of horse mushrooms (mg kg$^{-1}$ dry weight).
Each value is the mean of two replicate crops.

| Compost | Casing | | | | Mean |
|---|---|---|---|---|---|
| | A Normal | | B Low | | (LSD = 0.37) |
| | 1st Flush | 2nd Flush | 1st Flush | 2nd Flush | |
| A Normal | 1.28 | 2.91 | 3.25 | 4.40 | 2.96 |
| B Normal | 1.16 | 3.39 | 0.69 | 2.63 | 1.96 |
| C Low | 5.76 | 1.15 | 3.80 | 1.29 | 3.00 |
| D Low | 6.02 | 2.94 | 4.28 | 3.21 | 4.11 |
| Mean (LSD = 0.26) | 3.08 | | 2.94 | | |

**Discussion**

The main source of cadmium in the mushroom composts was poultry manure, and composts with lower cadmium contents were produced by using poultry manure and lime/chalk with a low cadmium content (0.24 mg kg$^{-1}$ dry weight) and/or alternative raw materials. However, the results of this work have shown that production of a lower cadmium content compost does not necessarily result in a lower cadmium content of horse mushrooms, and that compost pH may be as or more important than cadmium content in determining availability of cadmium to the mushroom. The pH of the compost declined during the cropping period, as occurs with *Agaricus bisporus* compost (Noble et al, 2008).

The lowest cadmium content mushrooms were grown on a poultry manure/ straw compost (B) having a pH of 7.41 at spawning and 6.85 at the end of cropping. A poultry manure/straw compost (A) with a higher cadmium content at spawning and lower pH at the end of cropping produced mushrooms with a higher cadmium content in both the first and second flushes. Composts prepared without poultry manure and with a lower pH and cadmium content at spawning (C and D) produced first flush mushrooms with high cadmium contents. However, these composts produced second flush mushrooms with a low cadmium content; presumably available cadmium had been released from the substrate in the first flush due to the low pH. The low pH of Compost C and D was probably due to the use of ammonium sulphate in the compost formulation. Cadmium concentrations in plants have also been shown to increase markedly as the soil pH decreases from 7.5 to 5 (He and Singh 1993; Singh et al. 1995).

The main source of cadmium in the mushroom casing was sugar beet lime, probably from the residual soil in the material. Casing with a low cadmium content was produced by using chalk in place of sugar beet lime. However, the casing had only a small effect on the cadmium content of the mushrooms compared with the effect of the compost, even though the ranges in cadmium contents of casings and composts examined was similar. The effect of the pH of the casing on the cadmium content of horse mushrooms was not examined in these experiments.

The cadmium contents of all the mushroom samples analysed were less than the new EU limit for cadmium in horse mushrooms (1 mg/kg fresh weight). However, only mushrooms grown on Compost B and Casing B were below the original limit of 0.2 mg/kg in both the first and second flushes (Table 8). From the results of this work, the critical cadmium contents in the substrates for achieving these low cadmium levels in the mushroom appear to be less than 0.3 mg/kg dry weight of compost or casing. These levels need to be combined with compost and casing pH values that do not fall below about 6.85 during cultivation. The effect of increasing the pH of the compost on the cadmium content and yield of horse mushrooms should be investigated. Increasing the compost pH could be achieved by adding urea, chalk ($CaCO_3$) and/or hydrated lime (CaOH) during the composting process, or by adding the latter two materials after Phase II compost is made.

Availability of cadmium from the compost or casing was unrelated to the yield of horse mushrooms. Compost prepared with vegetable waste produced a good yield and this should be investigated further. The wet peat + chalk casing produced a higher mushroom yield than the milled peat + sugar beet lime casing; however, the independent effects of peat types and lime or chalk sources and amounts on horse mushroom yield and cadmium content should be investigated further.

It is likely that substrate cadmium content and pH will influence the cadmium content of other mushrooms (e.g. *Agaricus blazei* or brozei and *Pleurotus* species) which have a tendency to accumulate heavy metals.

**Conclusions**

1. Poultry manure was the main source of cadmium in compost and sugar beet lime was the main source of cadmium in casing; composts and casing with low cadmium contents were prepared by using poultry manure and lime with a low cadmium content (0.24 mg $kg^{-1}$ dry weight) and/or by reducing or avoiding these raw materials.
2. Availability of cadmium in compost to horse mushrooms depended not only on the cadmium content of the compost, but also on its pH values at the start and end of cultivation; composts with a low cadmium content but also a low pH still produced horse mushrooms with a high cadmium content in the first flush.
3. The cadmium content of the casing layer had a smaller effect on the cadmium content of horse mushrooms than the cadmium content of the compost; reducing the cadmium content of the casing by replacing sugar beet lime with chalk resulted in a small reduction in the cadmium content of horse mushrooms.
4. The cadmium contents of all the first and second flush horse mushrooms in these experiments were below the new EU limit (1 mg $kg^{-1}$ fresh weight) irrespective of the compost and casing treatments.
5. To achieve the previous EU limit for cadmium levels in horse mushrooms (0.2 mg $kg^{-1}$ fresh weight), the critical cadmium content in the compost and casing was about 0.3 mg $kg^{-1}$ dry weight; this maximum cadmium level needed to be combined with compost and casing pH values that did not fall below about 6.85 during cultivation.
6. The best yields of horse mushrooms (145 g $kg^{-1}$ substrate in three flushes) were obtained using a compost containing vegetable waste and casing prepared from wet peat and chalk.

**Action points**

- Raw materials used for preparing horse mushroom substrates, particularly poultry manure and lime sources, should be routinely tested for cadmium content. The results of this work indicate that this should not exceed 0.3 mg $kg^{-1}$ dry weight.
- The effect of increasing the pH of the compost and casing on the cadmium content and yield of horse mushrooms and other exotic species should be investigated.

- Increasing the compost pH could be achieved by adding urea, chalk ($CaCO_3$) and/or hydrated lime (CaOH) during or after the Phase I composting process, or by adding the latter two materials after Phase II compost is made.
- Addition during or after the Phase I is likely to result in better mixing but more likely to interfere with compost being produced for *A. bisporus* production, which does not need the addition of these materials.
- The same techniques could also be investigated for substrate production of other mushroom species that have a tendency to accumulate heavy metals.
- The use of casing prepared using chalk in place of sugar beet lime, and the use of a higher casing pH (by addition of additional chalk or hydrated lime) should be tested commercially.

**Technology transfer**

- The Grower Summary from this report will be made available to growers and composters.
- The findings of this work will be presented in an article in HDC News.
- The findings will be presented to scientific and industry meetings if and when possible.

**References**

Amsing, J. (1983) Inventarisatie van lood, cadmium, kwik, arseen en zink in geteelde champignons (Agaricus bisporus) en compost. De Chamipgnoncultuur 27: 275 – 285.

Anonymous (2000) MAFF UK – Multi-element survey of wild edible fungi and blackberries. (sheet 199). www. Food.gov.uk/science/surveillance/maffinfo/2000/maff-2000-199.

Anonymous (2001) Commission regulation (EC) No. 466/2001 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities, Legislation 44, L77, 1 – 13.

Anonymous (2006) Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities, Legislation, L364, 5 – 24.

Anonymous (2006) Basic horticultural statistics for the UK, 2005. www.defra.gov.uk.

Fleckenstein, J., Grabbe, K. (1981) Quantitative Aufnahme von Schwermetallen aus kontaminierten Substraten des Pilzanbaus durch *Agaricus bisporus*. Mushroom Science XI: 35 – 46.

Fritsche, G. (1979) Tests on breeding with *Agaricus arvensis*. Mushroom Science 10: 91 – 101.

Fritsche, G. (1989) Ontwikkelingswerk met de Akkerchampignon (*Agaricus arvensis* Schaeffer ex Secr). de Champignoncultuur 33: 7 – 13.

He, Q. B. and Singh, B.R. (1993) Plant availability of Cd in soils: I. Extractability of Cd in newly- and long-term cultivated soils. Acta Agric. Scand. B. Soil and Plant Sci. 43, 134-141.

Kruse, H., Lommel, A. (1979) Zeitschrift für Lebensmittel Untersuchung und Forschung 168: 444 – 447.

Movitz (1980) Hoga halter kadmium i vildvaxande, svenska champinjoner. Var Foda 32: 270 – 278 (Cadmium and mercurry content of Swedish mushrooms).

Noble, R. (1995) Alternative *Agaricus*: the culture of *Agaricus* W4 and Agaricus arvensis. HDC Project M4b, 14pp.

Noble, R., Dobrovin-Pennington, A. (2004) Partial substitution of peat in mushroom casing with fine particle coal tailings. Scientia Horticulturae 104: 351 – 367.

Noble, R., Dobrovin-Pennington, A., Turnbull, W. (2008a) Mushrooms: Carbon and nitrogen sources for organic and odourless mushroom compost. Project M 43, Horticultural Development Council.

Noble, R., Dobrovin-Pennington, A., Kilpatrick, M., Lyons, G., Sharma, H.S. (2008) Improving the spawn-running of mushroom compost. In: ISMS Cape Town Proceeding.

Singh, B.R., Narwal, R.P. and Almas A., (1998) Residual effect of organic matter on cadmium uptake by plants and its distribution in soils. In Contaminated Soils. Third International Conf. on Geochemsitry of Trace Elements in the Environment. Ed. R. Prost, INRA, Paris.

Stelte, W., Wolf, U., Wunderle, K. (1983) Über den Gehalt an toxischen Schwermetallen in Zuchtchampignons (III). Der Champignon 263: 8 – 15.

Thomas, B., Roughan, J.A., Watters, E.D. (1972) Lead and cadmium content of some vegetable foodstuffs. Journal of the Science of Food and Agriculture 23: 1493 – 1498.

Woggon, H., Bickerich, K. (1978) Die Nahrung 22.3, K13 – K15.