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# Study of heavy metals in some cultivated and uncultivated mushrooms of Turkish origin

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Twenty-four different species of uncultivated mushrooms of Turkish origin and one cultivated mushroom (*Agaricus bisporus*) growing in soil composts with 16 different compositions were analysed spectrometrically for their heavy metal (Pb, Cd, Hg, Fe, Cu, Mn, and Zn) contents. The highest Pb levels were 2.35 mg kg$^{-1}$ for the species *Agaricus bitorquis* and 7.00 mg kg$^{-1}$ for the species of *Hypholoma fasciculare* growing in the vicinity of the road. The highest Cd level was 3.42 mg kg$^{-1}$ for the species of *Hydnum repandum*. In the wild mushrooms the highest Fe content was 93.6 mg kg$^{-1}$ for the species of *Bovista plumbea* and the highest Cu content was 51.0 mg kg$^{-1}$ for the species *Tricholoma terreum*. The highest Mn content was 35.9 mg kg$^{-1}$ for the species *Laccaria laccata* and the highest Zn content was 31.6 mg kg$^{-1}$ for the species of *Agaricus bitorquis*. © 1998 Elsevier Science Ltd. All rights reserved.

## INTRODUCTION

Growing interest in health-conscious diets makes edible mushrooms valuable raw materials. In general, their fruiting bodies contain about 39.9% carbohydrate, 17.5% protein, 2.9% fats, on a dry weight basis, with the rest constituting the minerals (Latiff *et al.*, 1996). Many investigations have dealt with the metal contents of mushrooms, especially edible ones (Surinrut *et al.*, 1987; Jain *et al.*, 1988; Yasui *et al.*, 1988). As a result of environmental effects, generalized standard analysis methods for heavy metals in animal and vegetal tissues are required for calibration purposes. In a large number of publications, data have already been given about the contents of heavy metals in mushrooms (cultivated and in wild) (Stegnar *et al.*, 1973; Stijve and Roshnik, 1974; Seeger, 1976; Seeger *et al.*, 1976; Stijve and Besson, 1976; Stijve, 1977; Gast *et al.*, 1988; Latiff *et al.*, 1996). Compared to green plants, mushrooms can build up large concentrations of some heavy metals such as Cd and Hg (Meisch *et al.*, 1977; Kuusi *et al.*, 1981; Tyler, 1982; Bargagli and Baldi, 1984). As these metals are well-known for their toxicity at low concentrations, a great deal of effort has been made to evaluate the possible danger to human health from the ingestion of mushrooms (Gast *et al.*, 1988).

Decomposition of mushroom samples is an important considerarion for combined analytical methods. In most cases, when using highly sensitive measuring methods, such as flame AAS, graphite furnace AAS, ICP-OES, ICP-MS or inverse voltametry, the sample is measured in an aqueous solution (Knapp, 1991). In an earlier investigation, Se contents of some cultivated mushrooms were determined by AAS using the hydride technique and the standard-addition procedure (Piepponen *et al.*, 1983). Contents of Cd, Cu, Pb and Zn were determined in wild growing mushrooms in polluted and unpolluted regions. Determinations of the heavy metal concentrations were performed with AAS using flame atomisation (Gast *et al.*, 1988). Hg in mushroom samples was determined by cold vapour atomic absorption spectrophotometry (CVAAS) using $NaBH_4$ as the reducing agent (Rincon-Leon and Zurera-Cosano, 1986). Al, Pb and Cd were determined using a carbon rod atomizer on AAS (Mandic *et al.*, 1992).

The concentrations of four heavy metals (Pb, Cd, Hg and Cu) in 149 samples of mushroom fruiting bodies, representing 11 species, mainly all edible, were determined by AAS (Kalac *et al.*, 1991). The methods used for trace element determination were stripping voltametry for Pb, Cd and Cu (Vydra *et al.*, 1977) and AAS for Mn, Fe, Zn, Ni and Co, using a Perkin Elmer model 400. The two methods have been described in detail by Guy and Chakrabarti (1977). Pb and Cd contents of fungi were determined using the flame technique of

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AAS (Liukkonen *et al.*, 1983). Hg contents of fungi and mushrooms were analysed by cold-vapour AAS (Kuusi *et al.*, 1981; Zurera-Cosano *et al.*, 1988). As and Cd contents of some common mushrooms were determined by ICP analysis (Vetter, 1994). Determinations of Hg content in mushroom species in the Cordova Area (Spain) were carried out on a Perkin–Elmer model 2380 AAS (Zurera-Cosano *et al.*, 1986). Analysis of Ag was performed using a Thermo Jarrel Ash Video 11E AAS with an oxidizing air acetylene flame and background correction of the deuterium lamp (Falandysz *et al.*, 1994). Hg contents of macrofungi were measured from a $HNO_3$–$H_2SO_4$ digest by cold vapour AAS, and Al, Cu, Mn, Fe and Zn were analysed after dry-ashing and digestion in HCl by flame AAS (Perkin–Elmer 360) (Kojo and Lodenius, 1989). In that study, NaCl was added before analysing Al.

The present study relates to the determination of Hg, Pb, Cd, Fe, Cu, Mn, and Zn contents by using an AAS method for the fruit bodies of cultivated and uncultivated mushrooms of Turkish origin. Hg, in the mushroom samples, was determined by CVAAS. A graphite furnace was used for the analysis of Pb and Cd. The other metals were determined in the flame medium.

## MATERIALS AND METHODS

In the experiments, 16 cultivated mushrooms and a total 96 samples of wild-growing mushrooms, corresponding to 24 different species were used. The samples of uncultivated mushroom were collected from Söğütlü and Yeşilyurt forestries in Trabzon (Turkey). Species include *Laccaria laccata, Agrocybe* sp., *Leccinum* sp., *Agaricus bitorquis, Tricholoma terreum, Morchella* spp., *Agrocybe cylindracea, Pleurotus ostreatus, Lactarius volemus, Cantharellus ferruginascens, Amanita rubescens, Russala obscura, Amanita excelsa, Agarigus silvicolla, Russula cyanoxantha, Russula laurocerasi, Boletus* sp., *Hydnum repandum, Lactarius* sp., *Russula* sp., *Lactarius piperatus, Bovista plumbea, Hypholoma fasciculare, Russula delica* and *Agaricus bisporus.* These samples were washed with demineralized water. Each sample was dried at 50°C overnight and crushed in a mortar with achate beaker and pestle.

Digestion of mushroom samples was performed using an oxi-acidic mixture of $HNO_3$:$H_2SO_4$:$H_2O_2$ (4:1:1) (12 ml for 2–4 g sample) and heating at 75°C for three hours. After cooling, 20 ml demineralized water was added, the digest was again heated up to 150°C for four hours and brought to a volume of 25 ml with demineralized water.

For analysis of mercury, the technique described was as follows: 0.5 g was taken from the dried homogenized sample and its digestion was carried out using 7 ml of a $HNO_3$:$H_2SO_4$:$H_2O_2$ acid mixture at a ratio of 4:1:1; digestion was done at 60°C in a thermostatic bath, being completed in about 1.5 h. For oxidation of the sample a solution of potassium permanganate at 6%, w/v, was used. The excess of permanganate was reduced with a solution of hydroxylamine sulfate (Hatch and Ott, 1968).

Pb and Cd levels in the mushroom samples were determined using a GBC 3000 graphite furnace for

**Table 1. Elemental analyses obtained in 24 species of mushrooms: sample nos selected; from Söğütlü (1–8), from Yeşilyurt (9–18), from the nearest vicinity of the Trabzon–Yomra highway (19–24) in Trabzon (Turkey) ($mg\,kg^{-1}$ dry weight)**

| No. | Species | Edibility | Pb | Cd | Hg | Fe | Cu | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *Laccaria laccata* | Edible | 1.47 | 1.57 | 0.170 | 72.1 | 23.2 | 35.9 | 27.7 |
| 2 | *Agrocybe* sp. | Edible | 1.78 | 1.68 | 0.319 | 48.5 | 11.8 | 3.10 | 21.2 |
| 3 | *Leccinum* sp. | Edible | 0.437 | 0.660 | 0.259 | 31.3 | 8.65 | 5.52 | 29.6 |
| 4 | *Agaricus bitorqius* | Edible | 2.35 | 3.08 | 0.362 | 87.8 | 38.9 | 16.2 | 31.6 |
| 5 | *Tricholoma terreum* | Edible | 0.697 | 0.785 | 0.217 | 37.04 | 51.0 | 10.8 | 16.8 |
| 6 | *Morchella* spp. | Edible | 0.792 | 2.38 | 0.362 | 36.9 | 13.2 | 9.66 | 19.4 |
| 7 | *Agrocybe cylindracea* | Edible | 0.448 | 1.94 | 0.457 | 39.8 | 13.8 | 3.32 | 20.3 |
| 8 | *Pleurotus ostreatus* | Edible | 0.118 | 0.551 | 0.311 | 48.6 | 5.00 | 10.3 | 19.3 |
| 9 | *Lactarius volemus* | Edible | 1.33 | 1.25 | 0.128 | 43.6 | 18.9 | 5.38 | 21.4 |
| 10 | *Cantharellus ferrugina* | Unknown | 1.58 | 2.56 | 0.280 | 57.4 | 9.86 | 10.1 | 21.2 |
| 11 | *Amanita rubescens* | Edible | 1.09 | 1.15 | 0.400 | 23.9 | 13.4 | 5.88 | 27.7 |
| 12 | *Russala obscura* | Unknown | 2.19 | 3.40 | 0.416 | 50.5 | 16.4 | 8.80 | 26.8 |
| 13 | *Amanita excelsa* | Edible | 1.76 | 2.75 | 0.104 | 70.8 | 21.1 | 4.58 | 32.6 |
| 14 | *Agaricus silvicolla* | Edible | 0.426 | 4.85 | 0.236 | 56.7 | 6.61 | 4.31 | 18.1 |
| 15 | *Russula cyanoxantha* | Edible | 2.04 | 3.15 | 0.130 | 51.4 | 14.7 | 13.0 | 25.7 |
| 16 | *Russula laurocerasi* | Unknown | 1.84 | 1.21 | 0.312 | 38.7 | 18.9 | 12.4 | 25.9 |
| 17 | *Boletus* sp. | Edible | 0.965 | 1.03 | 0.136 | 31.1 | 4.71 | 2.95 | 26.2 |
| 18 | *Hydnum repandum* | Edible | 2.45 | 3.42 | 0.614 | 72.5 | 5.15 | 21.6 | 17.1 |
| 19 | *Lactarius* sp. | Unknown | 3.21 | 1.16 | 0.418 | 60.5 | 13.3 | 6.28 | 29.2 |
| 20 | *Russula* sp. | Unknown | 3.13 | 1.16 | 0.236 | 60.6 | 13.4 | 6.60 | 32.3 |
| 21 | *Lactarius piperatus* | Edible | 3.49 | 0.877 | 0.530 | 45.1 | 17.6 | 7.70 | 25.6 |
| 22 | *Bovista plumbea* | Inedible | 5.95 | 1.79 | 0.618 | 93.6 | 28.1 | 0.684 | 41.6 |
| 23 | *Hypholoma fasciculare* | Poisonous | 7.00 | 1.34 | 0.468 | 55.6 | 5.56 | 6.00 | 17.9 |
| 24 | *Russula delica* | Edible | 4.89 | 2.01 | 0.216 | 54.5 | 10.8 | 12.1 | 19.3 |

AAS. Determination of heavy metal (Fe, Cu, Mn, Zn) contents was carried out with a GBC 905 model AAS using flame atomisation. For the determination of Pb and Cd contents, deuterium and Smith-Hieftje background correction have been used. The standard-addition procedure was used in all determinations.

The wavelength and slit values, as nm, used for the determination of Pb, Cd, Fe, Cu, Mn, and Zn were: 283.3 and 0.5, 228.8 and 0.5, 248.3 and 0.2, 324.7 and 0.5, 279.5 and 0.2, and 213.9 and 0.5, respectively.

## RESULTS AND DISCUSSION

The heavy metal contents of the twenty four selected species of uncultivated mushrooms are shown in Table 1. The heavy metal contents of the cultivated mushrooms (*Agaricus bisporus*) growing in the soil composts with different composition are given in Table 2.

As can be seen Table 1, the levels of Pb in the samples collected in the Söğütlü forestries as a whole ranged from 0.118 mg $kg^{-1}$ to 2.35 mg $kg^{-1}$. The sample showing the highest levels was *Agaricus biterquis* which reached up to 2.353 mg of Pb per kg of dry weight. In the mushrooms taken from Yeşilyurt district, the highest Pb concentration was 2.5 mg $kg^{-1}$ for the species of *Hydnum repondum*. In the samples selected in the vicinity of Trabzon–Yomra highway, the highest Pb concentration was found 7.00 mg $kg^{-1}$ for the species of Hypholoma fasciculare.

Pb concentrations in washed and unwashed wild mushrooms are given in Fig. 1. In the uncultivated mushrooms subjected to wash, the amount of Pb decreases by 68% on average. The lower proportion of Pb of the washed mushrooms might be the result of a high pollution of Pb in the air. It has been reported that Pb concentration may be high in samples grown in the vicinity of a road (Seeger, 1982). In addition, Pb contents of inedible and poisonous mushrooms are higher than those of edible ones.

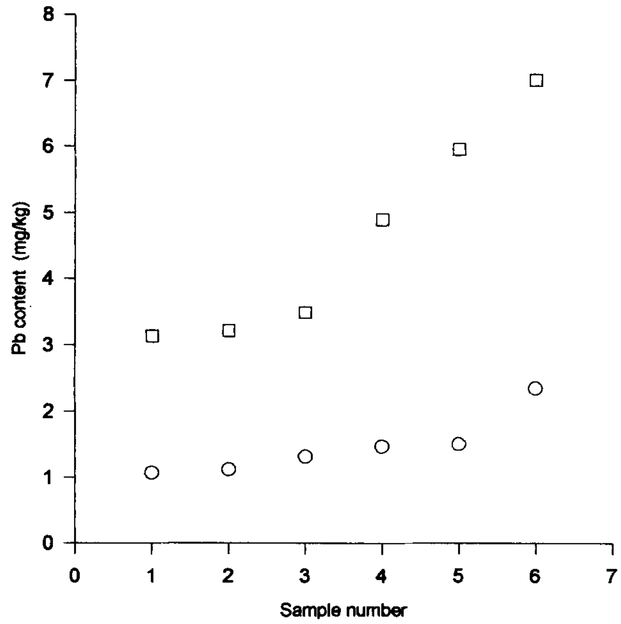


**Fig. 1.** Pb contents of washed and unwashed wild mushrooms. Symbols: □, unwashed mushrooms; ○, washed mushrooms.

In the cultivated mushrooms, the highest Pb concentration found was 0.878 mg $kg^{-1}$ grown in compost composed of 50:50 (w/w) of humus(Y) + perlite. In general, an increasing percent of perlite increases Pb concentration.

From Table 1, in the wild mushrooms supplied from Söğütlü forestries, the highest Cd content (3.08 mg $kg^{-1}$) found was for the species *Agaricus bitorquis*. Similar high values for Cd were in *Hydnum repandum* from Yeşilyurt and in *Russula delica* from the Trabzon-Yomra highway (3.42 ppm and 2.01 mg $kg^{-1}$, respectively.) From Table 2, in the cultivated mushrooms the

**Table 2. Elemental analyses obtained in cultivated mushroom (*Agaricus bisporus*) growing in soil composts with different composition (mg $kg^{-1}$ dry weight)**

| No. | Composition of soil | Pb | Cd | Hg | Fe | Cu | Mn | Zn |
|---|---|---|---|---|---|---|---|---|
| 1 | 80% Humus(Y) + 20%Perlite | 0.400 | 0.752 | 0.042 | 7.99 | 5.78 | 1.65 | 7.88 |
| 2 | 70% Humus(Y) + 30%Perlite | 0.611 | 0.764 | 0.048 | 8.00 | 5.44 | 1.68 | 8.73 |
| 3 | 60% Humus(Y) + 40%Perlite | 0.666 | 0.818 | 0.043 | 8.26 | 6.37 | 1.88 | 8.09 |
| 4 | 50% Humus(Y) + 50%Perlite | 0.878 | 0.914 | 0.089 | 10.3 | 6.09 | 1.90 | 8.26 |
| 5 | 100% Humus(Y) | 0.507 | 0.592 | 0.025 | 21.6 | 5.73 | 1.46 | 7.66 |
| 6 | 55%Humus(Y) + 40%Perlite + 5%Sand | 0.614 | 0.788 | 0.042 | 15.5 | 5.13 | 1.98 | 8.42 |
| 7 | 80% Garden soil + 20%Perlite | 0.415 | 0.614 | 0.061 | 11.7 | 6.03 | 2.6 | 12.2 |
| 8 | 100% Garden soil | 0.321 | 0.416 | 0.018 | 5.96 | 6.22 | 2.40 | 11.0 |
| 9 | 50% Humus(S) + 50% Perlite | 0.509 | 0.675 | 0.061 | 30.1 | 9.06 | 4.50 | 9.25 |
| 10 | 80% Humus(S) + 20% Perlite | 0.321 | 0.561 | 0.032 | 26.7 | 9.48 | 1.92 | 8.90 |
| 11 | 100% Humus(S) | 0.260 | 0.429 | 0.062 | 8.64 | 7.52 | 2.05 | 8.66 |
| 12 | 80% Humus(Y) + 20%Humus(S) | 0.402 | 0.869 | 0.041 | 11.2 | 7.20 | 1.89 | 9.70 |
| 13 | 97% Humus(Y) + 3%Sand | 0.565 | 0.964 | 0.032 | 31.4 | 6.42 | 2.73 | 10.5 |
| 14 | 80% Garden soil + 20%Sand | 0.206 | 0.462 | 0.049 | 20.3 | 6.75 | 2.51 | 10.3 |
| 15 | 80% Humus(S) + 20%Humus(G) | 0.408 | 0.890 | 0.068 | 23.0 | 8.27 | 3.09 | 10.3 |
| 16 | 80% Garden soil + 20%Humus(S) | 0.269 | 0.765 | 0.034 | 12.5 | 4.33 | 1.99 | 9.45 |
| | Average | 0.463 | 0.705 | 0.047 | 15.8 | 6.61 | 2.27 | 9.32 |

**Table 3. Statistical analysis results**

| Calculated values | Cu | Fe | Mn | Zn | Pb | Cd | Hg |
|---|---|---|---|---|---|---|---|
| Average, X(mg kg$^{-1}$) | 13.8 | 44.3 | 7.73 | 21.1 | 1.95 | 1.48 | 0.261 |
| Standard deviation, s | 0.484 | 0.117 | 0.086 | 0.068 | 0.045 | 0.071 | 0.015 |
| Relative s | 3.518 | 0.264 | 1.113 | 0.322 | 2.304 | 4.796 | 5.748 |
| Confidence interval | 13.759 ±0.601 | 44.340 ±0.145 | 7.726 ±0.107 | 21.92 ±0.084 | 1.953 ±0.056 | 1.477 ±0.088 | 0.261 ±0.019 |

For all experiments: $t = 2.776$ for $n = 5$.

highest Cd content found was 0.964 mg kg$^{-1}$ grown in compost composed of 97:3 (w/w) humus(Y) + sand. The present results are in agreement with values given in the literature (Liukkonen *et al.*, 1983; Kalac *et al.*, 1991; Vetter, 1993).

From Table 1, in the mushrooms selected from Söğütlü forestries, the highest Hg content found was 0.457 mg kg$^{-1}$ for the species *Agrocybe cylindracea*. Similar high values for Hg were 0.614 mg kg$^{-1}$ in *Hydnum repandum* (from Yeşilyurt) and 0.618 mg kg$^{-1}$ in *Bovista plumbea* (from the Trabzon-Yomra highway). From Table 2, in the cultivated mushrooms, the highest concentration of Hg found was 0.089 mg kg$^{-1}$ grown in the compost composed 50:50 (w/w) of humus(Y) + perlite. In an earlier investigation (Seeger, 1976), it was reported that Hg content (0.014–21.6 ppm) depended on the species of mushrooms. According to these data, Hg content found in our study is much lower than the highest value given in the literature.

In the wild mushroom samples, the highest Fe content was 93.6 mg kg$^{-1}$ for the species of *Bovista plumbea* (Table 1). In the cultivated mushrooms the highest concentration of Fe was 5.96 mg kg$^{-1}$ grown in garden soil (Table 2). It has been reported by Latiff *et al.* (1996) that Fe contents were 100–1216 mg kg$^{-1}$ in different species of mushrooms. Accordingly, Fe contents found in our study were much lower than those of given in the literature.

In the wild mushrooms samples, the highest Cu content was 51.0 mg kg$^{-1}$ for the species *Tricholoma terreum*, whereas the lowest Cu content was 5.00 mg kg$^{-1}$ in the species *Pleurotus ostreatus*. This result shows that the Cu content depends on the species of mushrooms.

The highest Mn content was 35.9 mg kg$^{-1}$ for the species *Laccaria laccata*, whereas the lowest Mn content was 2.95 mg kg$^{-1}$ for the species *Boletus*. According to these results, concentrations of Mn for mushrooms vary with species. In an earlier study, Mn contents in mushrooms were found to be 9.08 mg kg$^{-1}$ (Latiff *et al.*, 1996). The highest Zn content was 31.6 mg kg$^{-1}$, whereas the lowest Zn content was 17.1 mg kg$^{-1}$ in the species *Hydnum repandum*.

The results obtained show that the content of Hg differs according to the species analysed and the anatomical groups established. In a previous study (Zurera-Cosano *et al.*, 1986), there were found to be significant differences between species, although not between anatomical groups. The species factor for concentration of heavy metals has been pointed out by other authors (Bowen, 1966; Hopwood, 1975; Crowley, 1978). According to Stijve and Besson (1976), the mechanism by which some heavy metals are accumulated is somewhat obscure although it seems to be associated with a chelation reaction with the sulfhydryl groups of protein and especially with methionine. However, these same authors found very low levels of Pb, Cd and Hg in samples of *Psalliotabispora* cultivated with a high content of methionine.

The fact that toxic metals are present in high concentrations in the fruiting bodies of both edible and inedible fungi, from an area greatly favoured by mushroom pickers, is of particular importance in relation to the FAO/WHO (1976) Standards for Pb and Cd as toxic metals. The maximum permissible dose for an adult is 3 mg Pb and 0.5 mg Cd per week, but the recommended doses are only one-fifth of those quantities.

The results were verified by the UV vis spectrophotometric method of the O.A. chemists (Horwitz, 1970). The results obtained by AA and UV vis spectrophotometric methods were compared, and agreement was found (±6 percent).

The statistical analyses obtained from determinations of the heavy metal contents in the mushrooms of Turkish origin are given in Table 3.

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