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Heavy metals in edible mushrooms in Italy

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Abstract

The distribution of arsenic, cadmium, lead, mercury, and selenium was investigated in 1194 samples of 60 species of common, edible mushrooms collected mainly in the province of Reggio Emilia, Italy. The quantitative determination of heavy metals (mg/kg dry weight) was carried out by spectrophotometry, with the exception of Hg, which was determined by atomic absorption spectroscopy.

The amount of arsenic accumulated in the samples studied was in general modest. Sarcosphaera eximia, on the other hand, may contain arsenic concentrations reaching 1000 mg/kg dry weight. Within the Agaricus Subgenus Flavoagaricus, only Agaricus nivescens contains amounts of cadmium inferior to the allowed maximum level. The Cd levels in samples of Amanita caesarea, Boletus edulis and Boletus pinophilus exceeded the maximum amount allowed. The content of cadmium in Agaricus macrosporus is roughly 50 times the maximum weekly dose recommended by the WHO. The average amount of lead present in all samples, was in general, below the the maximum allowed concentration. Agaricus bitorquis, Agaricus arvensis, Agaricus essettei, Agaricus albertii, B. pinophilus, Clitocybe geotropa, and Macrolepiota rachodes had high contents of Hg that were within the range 5-10 mg/kg dry weight. Mushrooms in general, but species in the B. edulis group, in particular, were rich in selenium. Accumulation of specific heavy metals could be species-specific and thus assume a taxonomic role but it has proved in our study to be unreliable as an ecological indicator. $© 2005 Elsevier Ltd. All rights reserved.$

Keywords: Fungi; Edible mushrooms; Heavy metals; Ecology

1. Introduction

Mushrooms have been long known to accumulate high levels of heavy metals (e.g., [Allen & Steinnes, 1978; Coc](#page-6-0)[chi & Vescovi, 1996, 1997, 1997– 2005; Cocchi et al., 2002;](#page-6-0) [Dojmi Di Delupis & Dojmi Di Delupis, 1996; Kalac &](#page-6-0) [Svoboda, 2000; Mornand, 1990; Schmitt, 1987; Seeger,](#page-6-0) [1976a, 1976b Stegnar et al., Stegnar, Kosta, Byrne, &](#page-6-0) [Ravnik, 1973; Stijve & Besson, 1976](#page-6-0)). For instance, radioactive heavy metals in fruit bodies of edible mushrooms were already reported in the 1960s (Grüter, 1964). Several

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factors may affect the accumulation and concentration of trace elements and heavy metals in mushrooms. Concentrations of the elements are generally assumed to be species-dependent, but substrate composition is also considered to be an important factor ([Cocchi & Vescovi,](#page-6-0) [1997; Kalac & Svoboda, 2000; Stijve, Goessler, & Dupuy,](#page-6-0) [2004](#page-6-0)).

Intensive research has been carried out to detect and explain the presence and distribution of several heavy metals in edible mushrooms, in particular arsenic, cadmium, caesium, copper, iron, lead, manganese, mercury, selenium, rubidium, and zinc [\(Alonso, Salgado, Garcia,](#page-6-0) [& Melgar, 2000; Blanusa, Kucak, Varnai, & Saric, 2001;](#page-6-0) [Borella, Quaglio, Fabio, & Caselgrandi, 1992; Bressa,](#page-6-0) [Cima, & Costa, 1988; Falandysz, Gucia, Frankowska,](#page-6-0)

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[Kawano, & Skwarzec, 2001, 2002a, 2002b, 2002c, 2003,](#page-6-0) [2004; Ismail, 1994; Jorhem & Engman, 2000; Jorhem &](#page-6-0) [Sundstrom, 1995; Kalac, Burda, & Staskova, 1991; Ka](#page-6-0)[lac & Svoboda, 2000; Stijve, 2001; Stijve, Vellinga, &](#page-6-0) [Hermann, 1990; Svoboda & Kalac, 2003; Vetter, 1994\)](#page-6-0).

This study was started, on the one hand, to evaluate the possibility of using mushrooms as bioindicators of environmental contamination and, on the other, to assess the impact of the presence of heavy metals in mushrooms on public health. We report here on the distribution of arsenic, cadmium, lead, mercury, and selenium in a group of common, edible mushrooms collected mainly in the province of Reggio Emilia, Italy.

2. Materials and methods

2.1. Material examined

A list of all species studied is presented in Table 1. Most samples were collected in the region of Reggio Emilia, although some samples of Agaricus and Boletus (edulis group) from other geographic origins were also included. Samples were collected directly by two authors (LC and LV) or were provided for analysis by corresponding members of the Mycological Society, Bresadola. Chemical analyses were then carried out in the chemical laboratory of the Azienda Gas Acqua Consorziale (AGAC).

2.2. Chemical methods

Fruit bodes were first cleaned manually with soft brushes from soil and substrate and exsiccated in a ventilated oven on a plastic tray at $40-60$ °C over at least 24 h. The dry material was then stored, either intact or milled. Probes of the samples $(0.5-0.7g)$ were then put in Teflon containers, mixed with 10 ml $HNO₃$ and incubated for 30 min in a microwave oven (Perkin–Elmer Multiwave® microwave oven) at ≈ 180 °C at 30 bar pressure. The material obtained from this mineralisation was diluted with deionised H_2O to yield a sample of 50 ml. The quantitative determination of heavy metals (mg/ kg dry weight) was carried out using a ICP-OES Perkin– Elemer Optima 3000 XL spectrophotometer, with the exception of Hg, which was determined by atomic absorption spectroscopy using a cold-vapour Perkin–Elmer FIMS 100 ([Markert, 1992; Tsaler & Zaprianov,](#page-6-0) [1985\)](#page-6-0).

2.3. Statistical analysis

All values reported refer to the amount of heavy metals related to the dry weight, which is approximately one tenth of the fresh weight.

Subdivision Basidiomycotina Class Homobasidiomycetes Subclass Agaricomycetideae Bon Order Agaricales Clements (sensu stricto) Genus Agaricus L.: Fr. Subgenus Agaricus (L.: Fr.) Heinem. A. bisporus (J. Lange) Imbach (17 wild; 14 cultivated) A. bitorquis (Quél.) Sacc. (20) A. campestris L.: Fr. (30) Subgenus Flavoagaricus Wasser A. arvensis Schäff.: Fr. (23) A. essettei Bon (16) A. macrosporus (Møller and Schäff.) Pilát (16) A. nivescens Møller: Møller (17) A. sylvicola (Vitt.) Sacc. (10) Macrolepiota procera (Scop.: Fr.) Singer (15) Macrolepiota rachodes (Vitt.) Singer (10) Coprinus comatus (Müll.: Fr.) S. F. Gray (11) Order Amanitales Jülich Amanita caesarea (Scop.: Fr.) Pers. (7) Amanita ovoidea (Bull.: Fr.) Link (12) Amanita rubescens Pers.: Fr.(23) Amanita vaginata (Bull.: Fr.) Vitt. (15) Order Russulales (Roze) Kreisel Russula cyanoxantha (Schäff.) Fr. (29) Russula olivacea (Schäff.) Pers. (12) Russula vesca Fr. (25) Order Cortinariales Locquin ex Jülich Agrocybe aegerita (Briganti) Fayod (41) Cortinarius praestans (Cordier) Gillet (12) Rozites caperatus (Pers.: Fr.) P. Karsten (16) Order Entolomatales Locquin ex Jülich Clitopilus prunulus (Scop.: Fr.) Kümmer (14) Entoloma saundersii (Fries) Sacc. (23) Order Tricholomatales Kühner Armillariella mellea (Vahl: Fr.) Kümmer (14) Clitocybe geotropa (Bull.) Quél. (19) Clitocybe gibba (Pers.: Fr.) Kümmer (12) Clitocybe nebularis (Batsch.: Fr.) Kümmer (28) Hygrophorus penarius Fr. (15) Hygrophorus russula (Schäff.: Fr.) Quélet (11) Calocybe gambosa (Fr.: Fr.) Donk (18) Laccaria affinis (Sing.) Bon (17) Lepista nuda (Bull.: Fr.) Cooke (13) Lyophyllum decastes (Fr.: Fr.) Sing. (22) Marasmius oreades (Bolt.: Fr.) Fr. Order Boletales Gilbert Boletus aereus Bull.: Fr. (19) Boletus aestivalis (Paulet) Fries (24) Boletus edulis Bull.: Fr. (41) Boletus pinophilus Pilát and Dermek (31) Boletus edulis group (24 commercial samples) Boletus appendiculatus Schäff. (12) Boletus erythropus Pers. (10) Boletus luridus Schäff. (26) Suillus collinitus (Fr.) Kuntze (14) Suillus granulatus (L.: Fr.) Rouss. (17) Suillus luteus (L.: Fr.) Rouss. (17) Xerocomus chrysenteron (Bull.) Quél. (10) Xerocomus dryophilus (Thiers) Sing. (12)

Table 1 (continued)

The numbers in parentheses indicate the number of samples investigated.

Morchella semilibera De Cand.: Fr. (16) Ptychoverpa bohemica (Kromb.) J. Schroeter (19)

For each fungal species, descriptive statistics (minimum, mean, median, maximum, and standard deviation) were computed for the concentrations of arsenic, cadmium, lead, mercury and selenium. For graphical displays, boxplots, a graphical analogue of analysis of variance ([Tukey, 1977](#page-7-0)) were used. All statistical analyses were performed using Systat 11 (SPSS Inc., Chicago, IL, USA) on a PC running Windows XP.

3. Results and discussion

Out of approximately 7000 mushroom samples investigated during the whole period of the study, we chose 1194 samples in 60 mushroom species, for which at least 10 probes were available, in order to obtain representative statistical data.

[Table 2](#page-3-0) shows that the amount of arsenic accumulated in the samples studied is in general modest. Noteworthy, however, are Sarcosphaera eximia, with arsenic concentrations reaching 1000 mg/kg dry weight and Laccaria amethystina, two species for which we were able to examine only five samples each and which are accordingly not discussed in detail in this paper. The values we recorded, however, are in good agreement with the findings of [Stijve et al. \(1990\)](#page-6-0).

The amounts of cadmium recorded for species of the genus Agaricus [Subgenus Flavoagaricus Wasser $(=Flavescentes$ Møller and Schäff.), Amanita spp., and species in the Boletus edulis group are displayed in [Fig. 1](#page-4-0). The straight line drawn in all graphs represents the maximum concentration of cadmium (0.2 mg/kg fresh weight) allowed in cultivated mushrooms by the EU guideline 466/2001. Within the Agaricus Subgenus Flavoagaricus, only Agaricus nivescens contains amounts of cadmium inferior to the allowed upper level. Amanita caesarea exceeds, by approx. 4 times, the maximum amount allowed, and the values of cadmium present in both B. edulis and B. pinophilus are clearly above the upper allowed levels. The content of cadmium in Agaricus macrosporus (Møller and Schäff.) Pilát ($=A$ garicus alberti Bon), a common edible species, is noteworthy, as it is roughly 50 times the maximum weekly dose recommended by the WHO (0.5 mg) [\(Anon., 1996; WHO, 1996](#page-6-0)). [Table 3](#page-4-0) lists values measured for Cd (mg/kg dry weight) in vegetables commonly consumed in Italy. The concentration of Cd in species of Agaricus Subgenus Flavoagaricus is approximately 60 times higher than that contained in soybeans, the crop containing the highest Cd concentration. As approximately half of the maximum recommended Cd intake is provided by the normal alimentation, a weekly consumption of 50 g of fresh material of A. macrosporus, A. arvensis, Agaricus essettei, or Agaricus sylvicola would thus provide, at least theoretically, as mushrooms are not usually consumed daily, an amount of Cd already in excess of the maximum recommended weekly dose. It is also noteworthy that the average amount of cadmium contained in all samples studied is significantly higher than the maximum concentration allowed by the EU guideline 466/2001 ([Fig. 1](#page-4-0), [Table 2](#page-3-0)).

A similar picture can be seen for lead ([Fig. 2](#page-4-0), [Table](#page-3-0) [2\)](#page-3-0), although the average amount of lead contained in all samples is lower than the maximum allowed concentration. In particular, Calvatia utriformis contains high amounts of lead that are clearly above the maximum level allowed. Of all species included in [Table 1,](#page-1-0) only Agaricus bisporus (cultivated), Agaricus campestris, Armillariella mellea, the commercial Boletus edulis group (B. edulis, B. pinophilus, Boletus aereus, Boletus aestivalis), Boletus appendiculatus, Boletus erythropus, Boletus luridus, Cantharellus cibarius, Hydnum repandum, Clitocybe geotropa, Cantharellus gibba, Cantharellus nebularis, Clitopilus prunulus, Coprinus comatus, Entoloma saundersii, Hygrophorus penarius, Lyophyllum decastes, Marasmius oreades, Mitrophora hybrida, Morchella esculenta, Russula cyanoxantha, Russula olivacea, Suillus collinitus, Suillus granulatus, Suillus luteus, Xerocomus dryophilus and Xerocomus ferrugineus comply with the EU directive 466/2001 with regard to their lead content (3 mg/kg dry weight). All

Fig. 1. Boxplot displays of the content of cadmium in selected mushroom species of the genera Agaricus, Amanita, and Boletus. The straight line represents the maximum amount allowed by the EU directive 466/2001 (0.2 mg/kg dry weight, corresponding to approx. 2 mg/kg fresh weight). The dashed line shows the mean value of all samples studied.

others appear to contain lead levels that are either borderline (wild A. bisporus, A. bitorquis, Agrocybe aegerita, Amanita ovoidea, B. aereus, B. aestivalis, Calocybe gambosa, Cantharellus lutescens, Helvella crispa, Hirneola auricula-judae, Hygrophorus russula, Lepista nuda, Lycoperdon perlatum, Macrolepiota procera, M. rachodes, Ptycoverpa bohemica, Xerocomus chrysenteron, X. rubellus, X. subtomentosus) or clearly above the limits allowed (A. essettei, A. albertii, A. macrosporus, A. sylvicola, A. caesarea, A. rubescens, A. vaginata, Boletus edulis, B. pinophilus, C. utriformis, Cortinarius praestans, Laccaria affinis, Rozites caperatus, and Russula vesca). The case of C. utriformis is noteworthy, as all samples examined were collected, with no exceptions, in mountain meadows unlikely

Table 3 Cadmium concentration (mg/kg fresh weight) in vegetables

	Mean	Range
Wheat	0.068	$0.018 - 0.136$
Rice	0.11	$0.001 - 0.310$
Soy	0.17	$0.05 - 0.48$
Spinach	0.045	$0.019 - 0.070$
Lettuce	0.054	$0.031 - 0.147$
Cabbage	0.031	$0.022 - 0.094$
Peas	0.004	$0.003 - 0.005$
Beans	0.042	$0.019 - 0.075$
Potatoes	0.016	$0.005 - 0.055$
Onions	0.04	$0.01 - 0.09$
Peppers	0.029	$0.015 - 0.043$
Tomatoes	0.02	$0.01 - 0.08$
Apples	0.01	$0.005 - 0.027$
Pears	0.011	$0.010 - 0.013$
Oranges	0.002	$0.001 - 0.007$
Lemons	0.01	$0.01 - 0.04$

[AGAC, Internal report].

Fig. 2. Boxplot displays of the contents of lead in Auricularia auriculajudae, Helvella crispa, Lycoperdum perlatum, and Calvatia utriformis. The straight line represents the maximum amount allowed by the EU directive 466/2001 (0.3 mg/kg dry weight, corresponding to approx. 3 mg/kg fresh weight). The dashed line shows the mean value of all samples studied.

to be polluted. This raises some doubts about the use of mushrooms as bioindicators, but this question will be addressed elsewhere.

The maximum acceptable content of mercury in mushrooms has so far not been established. The WHO recommends the maximum weekly intake of mercury not to exceed 0.3 mg (0.2 mg methyl mercury, respectively). Inspection of [Table 2](#page-3-0) reveals that A. bitorquis, A. arvensis, A. essettei, A. albertii, B. pinophilus, C. geotropa, and M. rachodes all have high contents of Hg that are within a range of 5–10 mg/kg dry weight. [Fig. 3](#page-5-0) also shows that, in general, all mushroom species contain very high amounts of Hg, species in the genus Boletus, and in particular B. pinophilus being those with the highest values. Our data closely match reports by other authors, in particular those of [Alonso et al. \(2000\),](#page-6-0) [Bargagli \(1992\), Falandysz et al. \(2003\)](#page-6-0), and [Stijve and](#page-6-0) [Besson \(1976\)](#page-6-0).

Fig. 3. Bar chart comparing the content of mercury in groups of mushroom species investigated.

Fig. 4. Bar chart comparing the content of selenium in groups of mushroom species investigated.

Selenium is an element which plays an important role in human nutrition and metabolism: according to the European Scientific Committee on Foods (SCF) the Dietary Reference Intake is $55 \mu g$; the total intake should, in no case, exceed 100 µg daily. [Table 2](#page-3-0) and Fig. 4 reveal that mushrooms, in general, but species in the B. edulis group, in particular, are rich in selenium. For B. pinophilus, the ingestion of 10 g of fresh mushroom would already provide the body an amount of selenium above to the maximum recommended daily dose.

Overall, our study has shown that mushrooms, and among them some excellent edible species such as A. caesarea or the B. edulis group, can accumulate high amounts of heavy metals.

Our data on heavy metals confirm reports by [Blanusa](#page-6-0) [et al. \(2001\), Falandysz et al. \(2001, 2002a, 2002b, 2003,](#page-6-0) [2004\), Jorhem and Engman \(2000\)](#page-6-0) and [Stijve and Bes](#page-6-0)[son \(1976\)](#page-6-0) for mercury and cadmium, and by [Kalac](#page-6-0)

[et al. \(1991\)](#page-6-0) for lead, cadmium and mercury that suggest mushrooms are able to accumulate heavy metals in amounts that, after chronic consumption, could be harmful to humans. Several authors (e.g. [Bargagli,](#page-6-0) [1988; Cocchi & Vescovi, 2000; Gremigni, 1986; Kalac](#page-6-0) [& Svoboda, 2000](#page-6-0)) have suggested that mushrooms could be potential bioindicators of environmental pollution with heavy metals. Our data, as exemplified by C. utriformis and A. albertii, do not support this hypothesis. Additional analyses of the complete dataset examined (data not shown) do not indicate any bioindicator role of any of the fungi we studied. On the other hand, it appears that accumulation of specific heavy metals could be species-specific and thus assume a taxonomic role. Species-dependent concentrations of specific elements in the fruiting bodies have already been observed [\(Kalac](#page-6-0) [& Svoboda, 2000](#page-6-0)). Additional investigations, currently ongoing with our samples, will provide further information on this potentially useful taxonomic tool.

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