



## Bioconcentration of chromium in edible mushrooms: Influence of environmental and genetic factors



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### ABSTRACT

Chromium concentrations were determined in 167 samples of wild edible mushrooms, collected from three different sites (urban, traffic and pastureland areas) in Lugo (NW Spain). The hymenophore (H) and the rest of the fruiting body (RFB) were analysed separately. The analyses were performed using inductively coupled plasma optical emission spectrometry (ICP-OES). The highest mean chromium levels (mg/kg dry weight) of 3.5 and 8.0, 4.5 and 6.2, and 6.2 and 4.3 were found in *Lycoperdon utriforme*, *Coprinus comatus* and *Agaricus campestris* in H and RFB, respectively. The highest concentrations of chromium were observed in terrestrial saprophytic species in relation to mycorrhizal species. With respect to the underlying substrates, chromium concentration was lowest in the pastureland area (24.6 mg/kg dw). All mushroom species were bioexclusors of chromium ( $BCF < 1$ ) with statistically significant differences ( $p < 0.001$ ). The consumption of mushrooms harvested from the areas investigated poses no toxicological risk to human health due to chromium.

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### 1. Introduction

Mushrooms constitute part of the human diet because they provide nutrients: carbohydrates, proteins, vitamins and minerals (Kalač, 2009; Reis et al., 2012), nutraceuticals (Barros et al., 2008), and other compounds with antihyperglycemic and antioxidant activities (Ribeiro et al., 2007; Gursoy et al., 2009; Sarikurkcu et al., 2010; Liu et al., 2012). Moreover, mushrooms are considered a delicacy, they are part of the traditional culinary culture of some countries, they are generally consumed worldwide and, in some countries/regions, they are very popular (Falandysz and Borovička, 2013). Given their relative position in the food chain, the occurrence of high metal contents in mushrooms is considered important because the presence of some metals, mainly cadmium, mercury and lead, may constitute a possible toxicological hazard. Mushrooms that are grown in natural habitats, geochemically anomalous areas and anthropogenically polluted soils can uptake metals and concentrate them in the edible parts; thus, these toxic metals represent serious threats to the environment, animals and humans (Řanda and Kučera, 2004; Gursoy et al., 2009; Aloupi et al., 2012; Falandysz et al., 2012a,b). However, many elements, such as Fe, Zn, Mn, Cr and Se, are essential for human metabolism in low concentrations because they are enzyme activators. These elements become toxic as their concentration increases. The process of heavy-metal accumulation in mushrooms is species-dependent (Figueiredo et al., 2007; Isildak et al., 2004; Li et al., 2011; Falandysz et al., 2012c). Several

already reported studies have evaluated the contents of toxic metals in mushrooms to tentatively characterise pollution bioindicators through bioconcentration factors, to investigate the relationship between the metal concentration in mushrooms and in the underlying soil where mushrooms grow, or to evaluate metal intake through the consumption of contaminated mushrooms (Demirbaş, 2001; Sivrikaya et al., 2002; Zaichick, 2002; Mendil et al., 2004; Jonnalagadda et al., 2006; Dogan et al., 2006; Muñoz et al., 2006; Yamac et al., 2007; Falandysz et al., 2008, 2012a; Gucia et al., 2012a). Although the main factors that contribute to the accumulation of metals by mushrooms have not been well identified, the uptake of metals is determined by several conditions, specifically, environmental properties (e.g., the metal contents in soil, water and air, the pH and the substrate composition) and genetic properties (e.g., the ecology, the species and the morphological portion) (Falandysz et al., 2007; García et al., 2008; Campos et al., 2009; Melgar et al., 2009; Falandysz et al., 2012c; Jarzyńska and Falandysz, 2012a,b). For example, in contaminated aqueous solutions, pH was experimentally determined to be decisive in the biosorption of metals by *Agaricus macrosporus* (Melgar et al., 2007).

Chromium (Cr) is a metallic element that can exist in several oxidation states; the trivalent and hexavalent states are the most important biological forms. Chromium is the tenth most abundant element in the earth's mantle, and it is used extensively for several industrial purposes in both its tri- and hexavalent forms, depending upon the final use of the end product; these industrial applications include electroplating, timber treatment, pulp production, mineral ore, and petroleum refining (Wang et al., 2011). Chromium is ubiquitous in nature; it occurs in air, water, soil and

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biological materials. It is therefore considered a bioelement with important metabolic functions. Hexavalent chromium is highly toxic; it exhibits genotoxic and carcinogenic actions in both animals and humans (Macfie et al., 2010). In addition to the different biological and toxicological properties of the various chromium species, their mobilisation from soils and their uptake by vegetables also differ depending upon several factors, including the microbial activity and the organic contents in the soil.

Both Cr<sup>III</sup> and Cr<sup>VI</sup> are taken up by plants: Cr<sup>VI</sup> is taken actively by sulphate carriers and is immediately converted to Cr<sup>III</sup> in roots; Cr<sup>III</sup> is taken up passively and is retained by the ion-exchange sites of cell walls. Chromium concentrations in plants follow the general order: roots > leaves > fruits (Van Paemel et al., 2010). Moreover, whereas Cr<sup>III</sup> is strongly retained in soil particles, Cr<sup>VI</sup> is weakly adsorbed and is readily available for plant uptake (Figueiredo et al., 2007).

Feed and food contain chromium in both inorganic forms and organic complexes; however, the precise speciation of dietary chromium compounds is not known (EFSA, 2009).

All of the previous studies conducted on the Cr in mushrooms reported the total Cr content, although one group of authors did specifically analyse the content of Cr<sup>VI</sup> (Figueiredo et al., 2007).

The aim of the present investigation was to determine the accumulation capacity (concentration or exclusion) of total chromium in the fruiting bodies of some edible mushrooms (cultivated and wild species) in relation to various factors, including the growth substrate (metal content, acidity, and organic matter content), the species and ecology (mycorrhizal and saprophytic), and the morphological part (hymenophore and rest of the fruiting body). We also evaluated the contribution of mushrooms to the daily human intake of this trace metal.

## 2. Materials and methods

### 2.1. Sampling

Fruiting bodies and the underlying substrate were collected from three different sites: urban, traffic and pastureland areas in the province of Lugo (Galicia, NW Spain) during 2009 and 2010. The species were selected in relation to their culinary quality, commercialisation, and frequency in the areas of study.

In total, 167 samples of edible mushrooms belonging to 22 species of *Basidiomycetes* fungi (Table 1) were collected: 12 mycorrhizals, 8 saprophytes (terrestrial) and 2 saprophytes (lignicolous).

Simultaneously, the upper soil horizons (0–10 cm, after the superficial layer of organic detritus was removed) were also collected at appropriate sampling places. A total of 50 underlying soil samples, where mushrooms grow, were analysed.

These samples were cleaned (not washed), cut and separated into two parts: the hymenophore (H) and the rest of the fruiting body (RFB) (e.g., the cap, except the hymenophore, and the stalk).

Fresh mushrooms, after being cleaned of the plant and substrate debris with a plastic knife, were air-dried for several days and further dried in an oven at 50 °C until the samples reached a constant weight (approximately 40 h); the samples were subsequently pulverised in an agate mortar. Sub-samples (between 0.3 and 0.5 g) of powdered mushrooms were wet-digested with 8 ml of concentrated nitric acid (Suprapur, Merck) in closed PTFE vessels in a microwave oven (ETHOS 20, Milestone). The digested samples were diluted to a final volume of 50 ml with deionised water. All samples were analysed in triplicate (García et al., 2009).

Soil substrate samples were dried at room temperature for several weeks and then sieved through a pore size of 2 mm. A representative sample of up to 0.5 g was digested in 10 ml of concentrated nitric acid (Suprapur, Merck) for 10 min in a closed PTFE vessel in a microwave oven (ETHOS 20, Milestone). The extract obtained was filtered through Whatman No. 42 filter paper into a volumetric flask and was brought to a final volume of 50 ml with deionised water.

### 2.2. Analyses

The dissolved metals were analysed via inductively coupled plasma optical emission spectrometry (ICP-OES) on a Perkin Elmer OPTIMUM 4300 DV with the use of the WinLab32 software package. The wavelengths of 267.707, 205.562 and 283.552 nm were used for the analysis of chromium. The integration time was varied between 1 and 5 s, with three replicates per sample. The detection limit was 0.6 ng/kg.

**Table 1**

Chromium concentrations (mg/kg dw) in the analysed species of mushrooms. The number of samples (*n*), the parts, the mean concentrations, the standard deviations, the range, the BCF, and the coefficients of Pearson correlations (*r*) are indicated.

Species	<i>n</i>	Part	Mean ± SD	Range	BCF	<i>r</i>
<i>Agaricus campestris</i> L.	7	H	6.2 ± 5.0	1.8–16	0.24	0.398
		RFB	4.3 ± 2.4	1.7–8.7	0.22	0.080
<i>Agaricus urinascentis</i> (Jul. Schäff. & F.H. Moller) Singer	12	H	3.2 ± 0.86	2.3–5.5	0.21	0.019*
		RFB	3.5 ± 0.93	2.4–5.4	0.24	−0.156
<i>Agaricus silvicola</i> (Vittad.) Peck	5	H	4.5 ± 2.2	2.3–8.0	0.25	0.757
		RFB	3.3 ± 0.52	2.8–4.2	0.26	0.019*
<i>Agrocybe cylindracea</i> (DC.) Marie	4	H	2.2 ± 0.24	2.0–2.4	(1)	
		RFB	1.8 ± 0.26	1.4–2.0		
<i>Amanita rubescens</i> Pers.	11	H	2.5 ± 0.55	1.7–3.3	0.25	0.386
		RFB	3.6 ± 1.1	2.7–5.9	0.34	0.360
<i>Boletus aereus</i> Bull.	3	H	2.8 ± 0.29	2.5–3.1	0.06	0.803
		RFB	3.8 ± 0.27	3.5–4.0	0.09	0.414
<i>Boletus badius</i> (Fr.) Fr.	9	H	2.4 ± 0.70	1.6–3.8	0.21	0.717
		RFB	3.6 ± 0.57	2.7–4.9	0.33	0.598
<i>Boletus edulis</i> Bull.	10	H	2.8 ± 1.2	1.5–5.7	0.24	−0.235
		RFB	3.8 ± 1.2	1.1–5.3	0.32	−0.358
<i>Boletus pinophilus</i> Pilát & Dermek	9	H	4.0 ± 1.7	2.3–7.3	0.24	0.028*
		RFB	4.4 ± 2.8	2.0–11	0.22	0.530
<i>Boletus reticulatus</i> Schaeff.	3	H	3.8 ± 0.92	3.1–4.8	0.13	0.391
		RFB	3.0 ± 0.69	2.4–3.7	0.10	0.853
<i>Lycoperdon utriforme</i> Bull.	3	H	3.5 ± 1.7	2.2–5.4	0.21	0.046*
		RFB	8.0 ± 7.2	2.8–16	0.30	0.418
<i>Cantharellus cibarius</i> Fr.	11	H	0.67 ± 0.76	0.25–2.9	0.04	0.147
		RFB	0.78 ± 1.2	0.13–4.2	0.05	0.138
<i>Clitocybe nebularis</i> (Batsch) P. Kumm.	9	H	1.7 ± 1.0	0.28–2.6	0.09	0.330
		RFB	3.0 ± 2.3	0.20–6.4	0.12	0.641
<i>Coprinus comatus</i> (O.F. Müll) Pers.	7	H	4.5 ± 3.1	1.8–10	0.22	0.511
		RFB	6.2 ± 4.8	2.7–16	0.29	0.224
<i>Fistulina hepatica</i> (Schaeff.) With.	4	H	3.1 ± 1.2	2.2–4.8	(1)	
		RFB	3.9 ± 1.0	2.4–4.7		
<i>Hydnum repandum</i> L.	8	H	2.2 ± 0.84	1.1–3.3	0.20	0.043*
		RFB	2.6 ± 0.79	0.83–3.4	0.22	0.479
<i>Lactarius deliciosus</i> L. (Gray)	9	H	5.2 ± 4.4	1.3–15	0.53	−0.194
		RFB	3.4 ± 2.6	0.84–8.5	0.32	0.046*
<i>Leccinum scabrum</i> (Bull.) Gray	5	H	2.6 ± 0.72	1.8–3.7	0.25	−0.355
		RFB	3.1 ± 0.65	2.2–4.0	0.32	−0.587
<i>Lepista nuda</i> (Bull.) Cooke	9	H	3.6 ± 1.9	1.5–7.0	0.37	−0.434
		RFB	3.2 ± 1.8	2.0–7.6	0.31	−0.252
<i>Macrolepiota procera</i> (Scop.) Singer	10	H	2.9 ± 1.4	1.2–5.3	0.18	0.576
		RFB	2.3 ± 1.2	0.83–4.5	0.16	0.039*
<i>Russula cyanoxantha</i> (Schaeff.) Fr.	10	H	2.8 ± 2.7	0.71–9.1	0.11	0.499
		RFB	2.8 ± 2.7	1.1–9.8	0.15	0.171
<i>Tricholoma portentosum</i> (Fr.) Quéf.	9	H	0.97 ± 0.38	0.49–1.7	0.09	0.076
		RFB	0.80 ± 0.50	0.39–1.7	0.06	0.585

\* Significant difference, *p* < 0.05, (1) Wood-decaying and/or cultivated fungal species.

The argon flow was regulated to 15 ml/min, the sample flow was regulated to 1.5 ml/min, and the potential was 1300 W (García et al., 2008).

The accuracy of the method was evaluated using reagent blanks and standard reference materials. A reagent blank and standard reference material (Lichen, IAEA-336, International Atomic Energy Agency) were included to verify the accuracy and precision of the digestion and subsequent analysis procedure. Discrepancies between certified values and concentrations quantified were below 10% (3.93%). The results obtained for 10 replicate analyses of this standard material were:

Certified value: 1.06 mg/kg dw; range: 0.84–1.23.  
Measured value: 1.072 ± 0.23.  
Recovery (%): 106.3 ± 14.1.

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